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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07K 14/46, 19/00, C07H 21/04, A61K 38/17

A1

(11) International Publication Number: **WO 99/29726**

(43) International Publication Date: 17 June 1999 (17.06.99)

(21) International Application Number: PCT/AU98/00992

(22) International Filing Date: 30 November 1998 (30.11.98)

(30) Priority Data:
PP 0767 5 December 1997 (05.12.97) AU

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: PHOSPHOLIPASE INHIBITOR

(57) Abstract

The present invention relates generally to a broad–spectrum phospholipase enzyme inhibitor and uses therefor. More particularly, the present invention provides an inhibitor of phospholipase A_2 enzymes, wherein the inhibitor is a proteinaceous molecule including a peptide, polypeptide or protein which is derivable from the serum of a venomous animal. The present invention extends to derivatives, homologues, analogues, mimetics and functional chemical equivalents of the phospholipase A_2 inhibitor. The phospholipase A_2 inhibitor of the present invention is particularly useful in the production of a wide range of human and veterinary pharmaceutical products such as for the treatment of conditions involving phospholipase A_2 including, but not limited to, rheumatoid arthritis, osteoarthritis, asthma, allergic conditions, psoriasis, autoimmune disorders, inflammatory disease, multiple organ failure, acute pancreatitis, acute lung failure, septic shock, adult respiratory distress syndrome, insect and snake bite, amongst others.

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PHOSPHOLIPASE INHIBITOR

FIELD OF INVENTION

The present invention relates generally to a broad-spectrum phospholipase enzyme inhibitor and uses therefor. More particularly, the present invention provides an inhibitor of phospholipase A_2 enzymes, wherein the inhibitor is a proteinaceous molecule including a peptide, polypeptide or protein which is derivable from the serum of a venomous animal.

The present invention extends to derivatives, homologues, analogues, mimetics and functional chemical equivalents of the phospholipase A_2 inhibitor. The phospholipase A_2 inhibitor of the present invention is particularly useful in the production of a wide range of human and veterinary pharmaceutical products such as for the treatment of conditions involving phospholipase A_2 including, but not limited to, rheumatoid arthritis, osteoarthritis, asthma, allergic conditions, psoriasis, autoimmune disorders, inflammatory disease, multiple organ failure, acute pancreatitis, acute lung failure, septic shock, adult respiratory distress syndrome, insect and snake bite, amongst others.

BACKGROUND OF INVENTION

Bibliographic details of publications referred to by author in this specification are collected at end of the description.

Phospholipase A_2 (PLA_2) is a carboxylic acid esterase which removes the unsaturated fatty acid at the C-2 of glycerol.

PLA_2 enzymes comprise several sub-types including human Type I PLA_2, which is derived from human pancreas (Dennis, 1994; Dennis, 1997) and human Type II PLA_2 which is derived from human synovium. A Type III PLA_2 also exists.

Known PLA_2 enzymes are extremely stable to high temperatures and treatment with denaturing agents such as diethyl ether, chloroform or 8M urea, presumably due to their

compact structures.

Phospholipases, and in particular PLA_2, produce several adverse effects in humans and animals when administered, for example, in a venom, or when over produced by the body itself. For example, PLA_2 leads to the production of arachidonic acid which in turn may form arachidonic acid metabolites having proinflammatory activity (Flower *et al.*, 1979). Additionally, the PLA_2 in bee and snake venom is largely responsible for their toxicity to humans and animals.

Although many PLA_2 inhibitors are known, only a few proteinaceous PLA_2 inhibitors have been described. For example, a 344 amino acid protein has been obtained from the serum of mammals by enzymatic treatment. This molecule is useful in the treatment and diagnosis of inflammatory disease (United States Patent No. 5,344,764). Most PLA_2 inhibitors are synthetic chemical compounds possessing highly specific anti-inflammatory activity (e.g. ARL-67974, Astra; picolinic acid derivatives; thio-tetronic acid derivatives; 4-phenylakenoic and alkienoic acid PLA_2 inhibitors) or which prevent multiorgan failure.

Notwithstanding availability of some PLA_2 inhibitors, until advent of the present invention, all known PLA_2 inhibitors, including proteinaceous inhibitors, chemical compounds and small molecules, are limited in their range of inhibition. PLA_2 inhibitors which have previously been derived from snake venom, before instant invention, have also had a limited range of inhibition and are capable of inhibiting only snake PLA_2 enzymes. There is a need to identify PLA_2 inhibitors of general utility in the inhibition of PLA_2 enzyme activities.

In work leading up to the present invention, the inventors identified factors in the serum of snakes which protect the snakes against the toxic effects of their own venom or the venom of other animals. It has been surprisingly shown by the inventors that the main protective factor present in serum of snakes was capable of generally inhibiting PLA_2 enzymes. The purified PLA_2 inhibitor of the present invention provides a means for producing a wide range of pharmaceutical compounds of utility in the treatment of inflammatory conditions, autoinflammatory conditions, multiple organ failure, acute pancreatitis, acute lung failure,

septic shock, adult respiratory stress and the toxic effects of PLA_2 enzymes in insect and snake venoms, amongst other uses.

SUMMARY OF THE INVENTION

Abbreviations for phospholipase inhibitors are summarized in Table 1. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography. A summary of the SEQ ID NOs. is given in Table 2.

Throughout this specification, the term "at least" will be understood to mean that a stated integer or group of integers performs a stated function or is included in a stated composition of matter, but not to the exclusion of other functions or integers or groups of integers.

Throughout this specification, unless context requires otherwise, word "comprise", or variations such as "comprises", "at least comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

One aspect, an isolated molecule which is capable of inhibiting two or more phospholipase enzymes.

Another aspect of the present invention is directed to an isolated peptide, polypeptide or protein or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof which is capable of inhibiting two or more PLA_2 enzymes.

Yet another aspect of the present invention contemplates an isolated PLA_2 inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof from *Notechis scutatus* and is capable of inhibiting two or more of PLA_2 Type I, II and/or III enzymes. This molecule is referred to herein as "NSI".

Even yet another aspect of the present invention provides an isolated PLA_2 inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof from *Notechis ater* and is capable of inhibiting two or more of PLA_2 Type I, II and/or III enzymes. This molecule is referred to herein as "NAI".

Still yet another aspect of the present invention contemplates an isolated PLA_2 inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof from *Oxyuranus scutellatus* and is capable of inhibiting two or more of PLA_2 Type I, II and/or III enzymes. This molecule is referred to herein as "OSI".

Even yet another aspect of the present invention provides an isolated PLA_2 inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof from *Oxyuranus microlepidotus* and is capable of inhibiting two or more of PLA_2 Type I, II and/or III enzymes. This molecule is referred to herein as "OMI".

Another aspect of the present invention relates to an isolated PLA_2 inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof from *Pseudonaja textilis* and is capable of inhibiting two or more of PLA_2 Type I, II and/or III enzymes. This molecule is referred to herein as "PTI".

A further aspect of the present invention provides a PLA_2 inhibitor having a β-chain comprising an amino acid sequence substantially as set forth in one of SEQ ID NOs. 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 or 87 or an amino acid sequence having at least 40% similarity to one or more of the above listed sequences.

Yet a further aspect of the present invention contemplates a PLA_2 inhibitor having a β-chain encoded by a nucleotide sequence comprising a sequence as set forth in one of SEQ ID NOs. 8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89 or 90 or a nucleotide sequence having at least about 40% similarity to one or more of the above listed sequences or a nucleotide sequence capable of hybridizing under low stringency conditions at 42°C to one or more of SEQ ID NOs. 8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89 or 90.

Still yet another aspect of the present invention is directed to an isolated PLA_2 inhibitor comprising an α-chain comprising an amino acid sequence set forth in one of SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 or 85 or an amino acid sequence having at least about 40% similarity to one or more of the above listed sequences.

Another aspect of the present invention is directed to a PLA_2 inhibitor comprising an α- and β-chain wherein the α-chain comprises an amino acid sequence selected from SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 and 85 or an amino acid sequence having at least about 40% similarity to one or more of the above sequence and a β-chain comprising an amino acid sequence selected from SEQ ID NOs. 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 and 87 or an amino acid sequence having at least 40% similarity to one or more of the latter sequences.

A further aspect of the present invention provides a PLA_2 inhibitor comprising α- and/or β-chains having amino acid sequences or encoded by nucleotide sequence substantially as set forth for NSI, NAI, OSI, OMI and PTI in Table 2.

Accordingly, another aspect of the present invention contemplates a PLA_2 inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof comprising structure:

$$\alpha_m \beta_n$$

wherein

α is an α-chain of a PLA_2 inhibitor;

β is a β-chain of a PLA_2 inhibitor;

m is an integer from 0 to 10;

n is an integer from 0 to 10

with proviso that if m and n are not 0, then m>n and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α comprises an amino acid sequence selected from SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 and 85 or an amino acid

sequence having at least about 40% similarity to one or more of said sequences and β comprises an amino acid sequence selected from SEQ ID NOs: 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 and 87 or an amino acid sequence having at least about 40% similarity to one or more of said sequences. Preferably, m is 2-4 and n is 1-2. More preferably, m is 2 and n is 1.

A further aspect of the present invention contemplates a composition comprising an isolated or recombinant phospholipase inhibitor or a homologue, analogue, derivative, mimetic or chemical equivalent thereof together with one or more pharmaceutically acceptable carriers and/or diluents.

Another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides which encodes or is complementary to a sequence which encodes a phospholipase inhibitor or a homologue or derivative of said phospholipase inhibitor.

Another aspect of the present invention provides an isolated nucleic acid molecule having an α-chain encoded by a nucleotide sequence comprising as set forth in one of SEQ ID NOs. 5-7, 13-15, 39-41, 47-49, 54, 55, 60, 61, 67, 68, 75, 76, 82 or 88 or a nucleotide sequence having at least about 40% similarity to one or more of the above listed sequences or a nucleotide sequence capable of hybridizing to one or more of SEQ ID NOs. 5-7, 13-15, 39-41, 47-49, 54, 55, 60, 61, 67, 68, 75, 76, 82 or 88 under low stringency conditions at 42°C.

Yet another aspect of the present invention provides an isolated nucleic acid molecule having a sequence of nucleotides or complementary sequence of nucleotides comprising one or more of SEQ ID NOs. 5-8, 13-16, 39-42, 47-50, 54-56, 60-62, 67-70, 75-78, 82 to 84 or 88-90 or a nucleotide sequence having at least 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to any one or more of said sequences under low stringency conditions at 42°C.

Yet another aspect of the present invention provides a nucleic acid moelcule which encodes

an α-chain polypeptide of a PLA_2 inhibitor protein or a homologue or derivative thereof which nucleotide sequence has at least about 75% similarity to one or more of the nucleotide sequences set forth in SEQ ID NOs. 5-7, 13-15, 39-41, 47-49, 54-55, 60, 61, 67, 68, 75, 76, 82 or 88 or a nucleotide sequence capable of hybridizing under low stringency conditions at 42°C to one or more of said sequence.

Still yet another aspect of the present invention contemplates an isolated nucleic acid molecule which encodes a β-chain polypeptide or a PLA_2 inhibitor protein or a homologue or derivative thereof which has at least about 75% similarity to any one of the nucleotide sequences set forth in SEQ ID NOs:8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89 or 90 or a nucleotide sequence capable of hybridising under at least low stringency conditions at 42°C to one of said sequences.

Even yet another aspect of the present invention provides a nucleic acid molecule encoding a PLA_2 inhibitor having the structure:

$$\alpha_m\beta_n$$

wherein

α is an α-chain of a PLA_2 inhibitor;

β is a β-chain of a PLA_2 inhibitor; m is an integer from 0 to 10;

n is an integer from 0 to 10 with proviso that if m and n are not 0, then m>n and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α comprises an amino acid sequence selected from SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 and 85 or an amino acid sequence having at least about 40% similarity to one or more of said sequences and β comprises an amino acid sequence selected from SEQ ID NOs: 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 and 87 or an amino acid sequence having at least about 40% similarity to one or more of said sequences.

Still even yet another aspect of the present invention provides a nucleic acid molecule encoding a PLA_2 inhibitor having the structure:

$\alpha_m\beta_n$

wherein

α is an α-chain of a PLA_2 inhibitor;

β is a β-chain of a PLA_2 inhibitor; m is an integer from 0 to 10;

n is an integer from 0 to 10 with proviso that if m and n are not 0, then m>n and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α is encoded by an nucleotide sequence selected from SEQ ID NOs. 5-7, 13-15, 39-41, 47-49, 54, 55, 60, 61, 67, 68, 75, 76, 82 and 88 or a nucleotide sequence having at least about 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to one or more of said sequences under low stringency conditions at 42°C and β is encoded by a nucleotide sequence selected from SEQ ID NOs: 8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89, 90 or a nucleotide sequence capable of hybridizing to one or more of said sequences under low stringency conditions at 42°C.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1a is a graphical representation showing the elution profile for *N. scutatus* serum from A DEAE-Sephacel column. The SPP containing NSI elutes in P4.

Figure 1b is a graphical representation showing the further purification of SPP on a Mono-S column.

Figure 2a is a graphical representation showing the inhibition of snake venom PLA_2 enzymes with NSI, Day 1.

Figure 2b is a graphical representation showing the inhibition of snake venom PLA_2 enzymes with NSI, Day 2.

Figure 3a is a graphical representation showing the inhibition of non-snake venom PLA_2 by NSI, dilution group 1.

Figure 3b is a graphical representation showing the inhibition of non-snake venom PLA_2's by NSI, dilution group 2.

Figure 4 is a graphical representation showing the inhibition of $rhPLA_2$ by NSI.

Figure 5a is a graphical representation showing the pH stability of NSI.

Figure 5b is a graphical representation showing the temperature stability of NSI.

Figure 6a is a graphical representation showing the inhibition by deglycosylated NSI.

Figure 6b is a graphical representation showing the elution pattern for native (NSI) and deglycosylated NSI (DGNSI) from a size exclusion column. NSI elutes at 21-27 minutes.

Figure 7 is a graphical representation showing the Superose 12 elution profiles of SPP, notexin and SPP:notexin complex.

Figure 8 is a graphical representation showing the purification of endoproteinase Glu-C digestion products of the α-chain of NSI.

Figure 9 is a graphical representation showing the purification of trypsin digestion products of the α-chain of NSI.

Figure 10 is a graphical representation showing the purification of β-chain tryptic peptides. Peptides are marked T1 to T12 inclusive.

Figure 11 is a schematic representation showing the aligned amino acid sequences of phospholipase inhibitory α-chain polypeptides derived from *Notechis scutatus* (proseqnsi1; top row), coastal taipan *Oxyuranus scutellatus* (pseqct1; middle row) and inland taipan *Oxyuranus microlepidotus* (pseqit1; lower row). Numbers indicate the amino acid position in each sequence.

TABLE 1

ABBREVIATIONS	
NSI	PLA_2 inhibitor from *Notechis scutatus*
NSIαI	α-chain from isoform i of NSI
NSIαii	α-chain from isoform ii of NSI
NSIαiv	α-chain from isoform iv of NSI
NSI β	β-chain from NSI
NSIαiL	leader sequence of NSIαi
NSIαiiL	leader sequence of NSIαii
NSIαivL	leader sequence of NSIαiv
NSIβ	leader sequence of NSIβ
NAI	PLA_2 inhibitor from *Notechis ater*
NAIαi	α-chain from isoform i of NSI
NAIαii	α-chain from isoform ii or NSI
NAIαv	α-chain from isofrom v of NSI
NAIβ	β-chain from NAI
NAIαiL	leader sequence of NAIαi
NAIαiiL	leader sequence of NAIαii
NAIαvL	leader sequence of NAIαv
NAIβL	leader sequence of NAIβ
OSI	PLA_2 inhibitor from *Oxyuranus scutellatus*
OSIαi	α-chain from isoform i of OSI
OSIαii	α-chain from isoform ii of OSI
OSIβ	β-chain from OSI
OSIαiL	leader sequence of OSIαi

TABLE 1 (Continued)

OSIαiiL	leader sequence of OSIαii
OSIβ	leader sequence of OSIβ
OMI	PLA_2 inhibitor from *Oxyuranus microlepidotus*
OMIαi	α-chain of isoform i of OMI
OMIαii	α-chain of isoform ii of OMI
OMIβi	β-chain of isoform i of OMI
OMIβii	β-chain of isoform ii of OMI
OMIαiL	leader sequence of OMIαi
OMIαiiL	leader sequence of OMIαii
OMIβiL	leader sequence of OMIβi
OMIβiiL	leader sequence of OMIβii
PTI	PLA_2 inhibitor from *Pseudonaja textilis*
PTIαii	α-chain of isoform ii of PTI
PTIβi	β-chain of isoform i of PTI
PTIβii	β-chain of isoform ii of PTI
PTIαiL	leader sequence of PTIαi
PTIβiL	leader sequence of PTIβi
PTIβiiL	leader sequence of PTIβii

TABLE 2

SUMMARY OF SEQ ID NOS.

Description	SEQ ID NOs. Amino Acid Sequences	Nucleotide Sequences
NSIαi	1	5
NSIαii	2	6
NSIaiv	3	7
NSIβ	4	8
NSIαiL	9	13
NSIαiiL	10	14
NSIαivL	11	15
NSIβL	12	16
NAIαiN	17	-
NAIαiiN	18	-
NAIαed	19-23	-
NAIβN	24	-
NAIβed	25-34	-
NAIαi	35	39
NAIαii	36	40
NAIαv	37	41
NAIβ	38	42
NAIαiL	43	47
NAIαiiL	44	48
NAIαvL	45	49
NAIβL	46	50
OSIαi	51	54

TABLE 2 (continued)

OSIαii	52	55
OSIβ	53	56
OSIαiL	57	60
OSIαiiL	58	61
OSIβL	59	62
OMIαi	63	67
OMIαii	64	68
OMIβi	65	69
OMIβii	66	70
OMIαiL	71	75
OMIαiiL	72	76
OMIβiL	73	77
OMIβiiL	74	78
PTIαii	79	82
PTIβi	80	83
PTIβii	81	84
PTIαiiL	85	88
PTIβiL	86	89
PTIβiiL	87	90
Oligonucleotide primers	91	
	94	

Abbreviations for Table 2

NSI, PLA_2 inhibitor from *Notechis scutatus*

NAI, PLA_2 inhibitor from *Notechis ater*

OSI, PLA_2 inhibitor from *Oxyuranus scutellatus*

OMI, PLA_2 inhibitor from *Oxyuranus microlepidotus*

PTI, PLA_2 inhibitor from *Pseudonaja textilis*

α, α-chain of PLA_2 inhibitor

β, β-chain of PLA_2 inhibitor

i, ii, iv, v, indicates number of isoform

L, Leader sequence

αL, Leader sequence of α-chain

βL, Leader sequence of β-chain

βN, N-terminal region of β-chain

ed, Enzymatic digest

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides in one aspect, an isolated molecule which is capable of inhibiting two or more phospholipase enzymes.

The term "isolated" means that the molecule of the present invention is provided in a form which is distinct from that which occurs in nature, preferably wherein one or more contaminants have been removed. Accordingly, the isolated phospholipase inhibitor of the present invention may be partially-purified or substantially pure in which a substantial amount of the contaminants have been removed or the inhibitor may be in sequencably pure or substantially homogeneous form.

The term "sequencably pure" means that the isolated phospholipase inhibitor is provided in a form which is sufficiently purified to facilitate amino acid sequence determination using procedures known to those skilled in art.

The term "substantially homogeneous" includes an isolated phospholipase inhibitor of the present invention which is at least about 80% free of contaminants, more preferably at least about 90% free of contaminants, including 95-100% purity.

The preferred molecule of the present invention is a proteinaceous molecule such as but not limited to a peptide, polypeptide or protein. The present invention extends to recombinant, synthetic, derivative, homologue, analogue, mimetic and chemical equivalent forms of the molecule and all such forms of the molecule are encompassed by use herein of term "molecule" or "phospholipase inhibitor" or its abbreviation.

Preferably, the phospholipase is PLA_2.

Accordingly, in a preferred embodiment, there is provided an isolated peptide, polypeptide or protein or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof which is capable of inhibiting two or more PLA_2 enzymes. The peptide,

polypeptide or protein, recombinant, synthetic, derivative, homologue, analogue, mimetic and chemical equivalent forms of the PLA_2 inhibitor are all encompassed and included by use of the term "PLA_2 inhibitor" insofar as this term means the inhibitor of the present invention.

As exemplified herein, the present inventors have isolated and purified a PLA_2 inhibitor from serum of *Notechis scutatus, Notechis ater, Oxyuranus scutellatus, Oxyuranus microlepidotus* and *Pseudonaja textilis* by fractionation using anion exchange chromatography of dialysed serum and/or cation exchange chromatography. The present invention extends, however, to homologous PLA_2 inhibitors from other snakes and venomous animals, such as those snakes and animals listed below.

By "inhibiting" is meant that the enzyme activity of a phospholipase enzyme is reduced in the presence of the PLA_2 inhibitor of the present invention, compared to the activity of the phospholipase enzyme in the absence of the inhibitor.

Accordingly, an "inhibitor" or "inhibitory substance" is a substance, and in particular a peptide, polypeptide or protein, which is capable of inhibiting phospholipase enzyme activity.

The term "phospholipase inhibitor" or "PLA_2 inhibitor" or similar term shall be taken to refer to a peptide, polypeptide or protein or aggregates thereof such as a dimer or other multimer, fusion molecules or a homologue, analogue, mimetic, derivative or chemical equivalent thereof which is capable of inhibiting catalytic activity of a phospholipase enzyme, such as PLA_2 enzyme and in particular two or more PLA_2 enzymes.

For present purposes, a "phospholipase inhibitor" or "PLA_2 inhibitor" or similar term shall also be taken to include any peptide fragments or parts derived from a peptide, polypeptide or protein, aggregate or fusion molecules thereof or homologues, analogues, mimetics or chemical equivalents thereof, which, although they may have no inhibitory activity are at least useful as, for example, markers for antibody production, diagnostic markers, antagonists or for other embodiments herein described.

A "PLA_2 inhibitor" or "PLA_2 inhibitory protein" shall be taken to refer to a phospholipase inhibitor as hereinbefore defined which at least inhibits a PLA_2 enzyme but preferably more than one type of PLA_2 enzyme.

By "type of phospholipase [or PLA_2] enzyme" is meant a specific phospholipase such as a PLA_1, PLA_2, PLB, PLC or PLD enzyme. Preferably, it means more than one isoform of enzyme, for example, and in a most preferred embodiment, the Type I, Type II or Type III PLA_2.

In this regard, the inhibitory activity of the molecule of the present invention may be determined according to any standard method known to those skilled in art, such as by assaying for phospholipase enzyme activity in presence of the inhibitor as herein described or exemplified.

Those skilled in art will be aware that the amount of phospholipase inhibitor which is required to achieve inhibition may vary depending upon phospholipase enzyme being inhibited and/or the presence of other substances which may interfere with phospholipase activity inhibitor function.

In a preferred embodiment of invention, PLA_2 inhibitor described herein is capable of inhibiting at least 20%, more preferably at least about 50-70% and even more preferably at least about 80% of the PLA_2 activity present in a biological sample such as venom, synovial membrane, pancreas, skin, lung or other tissue or in association with an autoimmune response or inflammatory response such as an allergic reaction, rheumatoid arthritis, osteoarthritis, asthma, psoriasis, acute pancreatitis, multiple organ failure, acute lung failure, septic shock or adult respiratory distress syndrome, amongst others.

In particular, the phospholipase inhibitors of the present invention exemplified herein have been shown by the inventors to inhibit all groups of PLA_2 enzymes against which it has been tested. Additionally, the PLA_2 inhibitors of the present invention are capable of forming stable complexes with notexin (a purified PLA_2 enzyme) as judged by elution from a size

exclusion column and also prevents radioiodinated notexin from binding to isolated rat brain synaptosomes. The significance of these novel features is that these PLA_2 inhibitors can be used to treat many different conditions where PLA_2 enzymes are implicated or known to act.

As stated above, the preferred phospholipase enzyme which is inhibited by the inhibitor of the present invention is phospholipase A_2 (PLA_2). More preferably, the PLA_2 enzyme is a Type I, II or III phospholipase PLA_2 enzyme.

Accordingly, another aspect of the present invention provides an isolated PLA_2 inhibitor, as hereinbefore defined, capable of inhibiting two or more of PLA_2 Type I, II and/or III enzymes.

In one embodiment of the invention, the PLA_2 inhibitor is derived from the serum of an animal such as a snake or other reptile which produces a venom having toxic PLA_2 activity in humans or other animals.

The term "derived from" shall be taken to refer to the origin of an integer or group of integers from a specified source, but not to the exclusion of another possible source or sources of the integer or group of integers.

In a particularly preferred embodiment of invention, the PLA_2 inhibitor is derived from a snake.

In a preferred embodiment, the present invention provides an isolated PLA_2 inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof from *Notechis scutatus* and is capable of inhibiting two or more of PLA_2 Type I, II and/or III enzymes. This molecule is referred to herein as "NSI".

In another preferred embodiment, the present invention provides an isolated PLA_2 inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof from *Notechis ater* and is capable of inhibiting two or more of PLA_2 Type I, II and/or

III enzymes. This molecule is referred to herein as "NAI".

In yet another embodiment, the present invention provides an isolated PLA_2 inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof from *Oxyuranus scutellatus* and is capable of inhibiting two or more of PLA_2 Type I, II and/or III enzymes. This molecule is referred to herein as "OSI".

In still yet another aspect, the present invention provides an isolated PLA_2 inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof from *Oxyuranus microlepidotus* and is capable of inhibiting two or more of PLA_2 Type I, II and/or III enzymes. This molecule is referred to herein as "OMI".

Yet a further aspect of the present invention provides an isolated PLA_2 inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof from *Pseudonaja textilis* and is capable of inhibiting two or more of PLA_2 Type I, II and/or III enzymes. This molecule is referred to herein as "PTI".

The present inventors have determined that NSI is composed of two polypeptide chains, one 30 kDa glycosylated chain (α-chain) and one 25 kDa non-glycosylated chain (β-chain), as determined using denaturing SDS/polyacrylamide gel electrophoresis (SDS/PAGE). By mass spectrometry, the estimated molecular weights of the α-chain and β-chain are 22,604 and 19,817 Da, respectively. The α-chain exists in a number of isoforms (at least 3) that vary in amino acid sequence and/or glycosylation pattern. These chains combine in a 2:1 ratio to form the intact NSI complex that has a molecular weight of 110 kDa as judged by size exclusion chromatography. The glycosylation of the α-chain does not affect inhibition of PLA_2 enzymes. Similar ratios and polypeptide structures apply in relation to NAI, OSI, OMI and PTI.

The present invention clearly extends to all such isoforms of NSI, NAI, OSI, OMI and PTI and reference to these molecules includes reference to all isoforms and their recombinant, synthetic, derivative, homologue, analogue, mimetic and chemical equivalent forms.

The present invention extends further to a PLA_2 inhibitor which is capable of inhibiting a phospholipase enzyme wherein said molecule is capable of binding to the active site of a PLA_2 enzyme.

In a particularly preferred embodiment, the molecules according to this embodiment are capable of forming an interactive site with a phospholipase enzyme to inhibit the activity of enzyme. A "PLA_2 enzyme" includes one or more of PLA_2 Type I, II and/or III.

As used herein, the term "interactive site" shall be taken to refer to the primary, secondary or tertiary structure of a phospholipase inhibitor of the present invention which is in physical relation with a phospholipase enzyme wherein said physical relation is required for inhibitory activity of said inhibitor, or at least contributes to the inhibitory activity of said inhibitor.

In a more preferred embodiment, a molecule which is capable of forming an interactive site with a phospholipase enzyme mimics the 3-dimensional structure (i.e. tertiary structure) of NSI, NAI, OSI, OMI and/or PTI and, as a consequence, is capable of reproducing PLA_2 inhibitor:PLA_2 inhibitory interaction.

In this regard, whilst not being bound by any theory or mode of action, the mechanism of interaction between the PLA_2 inhibitor and the PLA_2 enzyme at least appears to be unique compared to the mode of interaction of other PLA_2 inhibitors with specific enzymes which they inhibit, thereby accounting for the generality of the PLA_2 inhibitory activity of the present invention. Those skilled in the art will be aware that once the structure of the interactive site between a PLA_2 inhibitor and a PLA_2 enzyme is established by standard X-ray crystallographic procedures, it is possible to synthesize peptides or other molecules (mimotypes or mimetics) which are capable of reproducing the inhibitory function of the inhibitors. Such mimotypes, whilst capable of forming a complex with interactive site with a phospholipase enzyme may not comprise the same amino acid sequence (i.e. primary structure) as the inhibitor α-chain and/or β-chain polypeptide(s), particularly in light of the finding by the inventors that both α-chain and β-chain polypeptides of the subject PLA_2 inhibitors are required for full inhibitory activity against the PLA_2 enzymes. Furthermore,

those skilled in the art will be aware that mimotypes may also comprise synthetic molecules such as chemical compounds or anti-idiotypic antibodies of the phospholipase inhibitor of the present invention capable of forming an interactive site with a phospholipase. Those skilled in the art will also be aware that mimotypes may be presented on a carrier molecule or embedded therein, such that the mimotype moiety is presented in a functional conformation capable of inhibiting the phospholipase activity. Accordingly, the present invention clearly extends to any molecule or composition of matter which at least comprises a mimotype or mimetic of NSI, NAI, OSI, OMI and/or PTI or the interactive site thereof.

Carrier molecules for presenting a mimotype may comprise the amino acid sequences presented as an in-frame fusion polypeptide with a polypeptide mimotype or alternatively, associated with a polypeptide mimotype by means of a disulfide bridge or other covalent bond formation, van der Waals interaction or ionic interaction, amongst others. Alternatively, wherein the mimotype moiety is a chemical compound, the mimotype may be embedded into a polypeptide carrier by any means known to those skilled in art.

Carrier molecules for presenting a mimotype may also comprise polysaccharide molecules, nucleic acid molecules such as RNA or DNA, biologically inert carriers such as tungsten or gold, amongst others, polymers such as starches, dextrans, glycogen, Percoll (Trademark of Pharmacia Fine Chemicals) or Ficoll (Trademark of Pharmacia Fine Chemicals), amongst others, agarose, polyacrylamide or carriers known to those in the pharmaceutical and/or biomolecular engineering industries.

In a particularly preferred embodiment, the present invention provides a PLA_2 inhibitor having a β-chain comprising an amino acid sequence substantially as set forth in one of SEQ ID NOs. 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 or 87 or an amino acid sequence having at least 40% similarity to one or more of the above listed sequences.

In a related embodiment, the present invention contemplates a PLA_2 inhibitor having a β-chain encoded by a nucleotide sequence comprising a sequence as set forth in one of SEQ ID NOs. 8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89 or 90 or a nucleotide sequence having

at least about 40% similarity to one or more of the above listed sequences or a nucleotide sequence capable of hybridizing under low stringency conditions at 42°C to one or more of SEQ ID NOs. 8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89 or 90.

Another aspect of the present invention is directed to an isolated PLA_2 inhibitor comprising an α-chain comprising an amino acid sequence set forth in one of SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 or 85 or an amino acid sequence having at least about 40% similarity to one or more of the above listed sequences.

In a related aspect of the present invention, there is provided an isolated PLA_2 inhibitor having an α-chain encoded by a nucleotide sequence comprising as set forth in one of SEQ ID NOs. 5-7, 13-15, 39-41, 47-49, 54, 55, 60, 61, 67, 68, 75, 76, 82 or 88 or a nucleotide sequence having at least about 40% similarity to one or more of the above listed sequences or a nucleotide sequence capable of hybridizing to one or more of SEQ ID NOs. 5-7, 13-15, 39-41, 47-49, 54, 55, 60, 61, 67, 68, 75, 76, 82 or 88 under low stringency conditions at 42°C.

Preferably, the PLA_2 inhibitors according to these aspects of the present invention are capable of inhibiting more than one type of PLA_2 enzyme and in particular two or more of PLA_2 Type I, II and/or III enzymes.

The term "similarity" as used herein includes exact identity between compared sequences at nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each or at the structural, functional, biochemical and/or conformational levels. In a particularly preferred embodiment, nucleotide and sequence comparisons are made at level of identity rather than similarity. Any number of programs are available to compare nucleotide and amino acid sequences. Preferred programs have regard to an appropriate alignment. One such program is Gap which considers all possible alignment and gap positions and creates an alignment with largest

number of matched bases and fewest gaps. Gap uses the alignment method of Needleman and Wunsch (1970). Gap reads a scoring matrix that contains values for every possible GCG symbol match. GAP is available on ANGIS (Australian National Genomic Information Service) at website http://mel1.angis.org.au..

The present invention further extends to hybrid PLA_2 inhibitors.

Accordingly, another aspect of the present invention is directed to a PLA_2 inhibitor comprising an α- and β-chain wherein the α-chain comprises an amino acid sequence selected from SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 and 85 or an amino acid sequence having at least about 40% similarity to one or more of the above sequence and a β-chain comprising an amino acid sequence selected from SEQ ID NOs. 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 and 87 or an amino acid sequence having at least 40% similarity to one or more of the latter sequences.

In one embodiment, the α- and β-chain are as occurring in the native PLA_2 inhibitor. In another embodiment, the α-chain of one PLA_2 inhibitor is associated with a β-chain of another PLA_2 inhibitor.

In a particularly preferred embodiment, the present invention provides a PLA_2 inhibitor comprising α- and/or β-chains having amino acid sequences or encoded by nucleotide sequence substantially as set forth for NSI, NAI, OSI, OMI and PTI in Table 2.

Preferred percentage similarities include at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90% or above such as 92%, 93%, 94% or 95-100%.

The present invention clearly extends to the full-length amino acid sequences of both the precursor and mature α-chains and β-chains of NSI, NAI, OSI, OMI and PTI and to heteropolymers and recombinant and isolated forms thereof, including fusion molecules.

In the present context, "homologues" of a phospholipase inhibitor or a PLA_2 inhibitor include those molecules which have a similar inhibitory activity to one or more of NSI, NAI, OSI, OMI and PTI, such as molecules having at least about 40% similarity to NSI, NAI, OSI, OMI or PTI at the amino acid or nucleotide level. Homologues may comprise fusion polypeptides between α-chains and β-chains with or without additional "spacer" sequences therebetween to facilitate folding and the ability of the fusion polypeptide to form an interactive site with a phospholipase enzyme. A homologue may be isolated or derived from the same species as the particular PLA_2 inhibitor or from a different species.

Furthermore, the amino acids of a homologous polypeptide may be replaced by other amino acids having similar properties such as, for example, hydrophobicity, hydrophilicity, hydrophobic moment, charge or antigenicity, and so on.

"Analogues" encompass PLA_2 inhibitors which are at least about 40% similar to one or more of NSI, NAI, OSI, OMI and/or PTI or their interactive sites, notwithstanding the occurrence of any non-naturally occurring amino acid analogues therein. "Analogues" also encompass polypeptide mimetics or mimotypes of the phospholipase inhibitor herein described.

The term "derivative" in relation to a PLA_2 inhibitor shall be taken to refer herein to mutants, parts or fragments derived from a functional PLA_2 inhibitor or homologues or derivatives thereof which may or may not possess PLA_2 inhibitory activity. Derivatives include modified molecules in which ligands are attached to one or more of the amino acid residues contained therein, such as carbohydrates, enzymes, proteins, polypeptides or reporter molecules such as radionuclides or fluorescent compounds. Glycosylated, fluorescent, acylated or alkylated forms of the subject PLA_2 are particularly contemplated by the present invention. Additionally, derivatives of a PLA_2 inhibitor which comprise fragments or parts of an amino acid sequence disclosed herein are within the scope of the present invention, as are homopolymers and heteropolymers comprising two or more copies of the subject polypeptides. Procedures for derivatizing peptides, polypeptides and proteins are well-known in art.

Particularly preferred analogues and derivatives of the PLA_2 inhibitors exemplified herein comprise an amino acid sequence which is capable of binding to the active site of a phospholipase enzyme and/or capable of forming an interactive site with a phospholipase enzyme.

Substitutions which may be included in a homologue, analogue or derivative of a subject PLA_2 inhibitor encompass amino acid alterations in which an amino acid is replaced with a different naturally-occurring or a non-conventional amino acid residue. Such substitutions may be classified as "conservative", in which case an amino acid residue contained in a phospholipase inhibitory protein is replaced with another naturally-occurring amino acid of similar character, for example, the substitutions Gly↔Ala, Val↔Ile↔Leu, Asp↔Glu, Lys↔Arg, Asn↔Gln and Phe↔Trp↔Tyr.

Substitutions encompassed by the present invention may also be "non-conservative", in which an amino acid residue which is present in a phospholipase inhibitor is substituted with an amino acid having different properties, such as a naturally-occurring amino acid from a different group (eg. a substitution of a charged or hydrophobic amino acid with alanine), or alternatively, in which a naturally-occurring amino acid is substituted with a non-conventional amino acid.

Amino acid substitutions are typically of single residues, but may be of multiple residues, either clustered or dispersed.

Naturally-occurring amino acids include those listed in Table 3. Non-conventional amino acids encompassed by invention include, but are not limited to those listed in Table 4.

Amino acid deletions will usually be of the order of about 1-10 amino acid residues, while insertions may be of any length. Deletions and insertions may be made to N-terminus, C-terminus or be internal deletions or insertions. Generally, insertions within the amino acid sequence will be smaller than amino-or carboxyl-terminal fusions and of the order of 1-4 amino acid residues.

The phospholipase inhibitor protein of the present invention or a homologue thereof may comprise polypeptide chains having an estimated molecular weight of from about 10 kDa to about 50 kDa as determined by SDS/PAGE or by mass spectrometry.

When the phospholipase inhibitor is in multimeric form, such as a heteropolymer of α-chain and β-chain polypeptides, it is also preferred that it exist as a trimeric protein having a molecular weight in the range of from about 30 kDa to about 150 kDa, more preferably about 45 kDa to about 90 kDa.

In a particularly preferred embodiment of the present invention, the phospholipase inhibitor or a homologue or analogue thereof is a heterotrimeric α_2:β protein.

Accordingly, another aspect of the present invention contemplates a PLA_2 inhibitor or a recombinant, synthetic, derivative, homologue, analogue, mimetic or chemical equivalent thereof comprising structure:

$$\alpha_m \beta_n$$

wherein

α is an α-chain of a PLA_2 inhibitor;

β is a β-chain of a PLA_2 inhibitor;

m is an integer from 0 to 10;

n is an integer from 0 to 10

with proviso that if m and n are not 0, then m>n and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α comprises an amino acid sequence selected from SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 and 85 or an amino acid sequence having at least about 40% similarity to one or more of said sequences and β comprises an amino acid sequence selected from SEQ ID NOs: 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 and 87 or an amino acid sequence having at least about 40% similarity to one or more of said sequences. Preferably, m is 2-4 and n is 1-2. More

preferably, m is 2 and n is 1.

In one embodiment, α and β are from the same PLA_2 inhibitor.

In another embodiment, α and β are from different PLA_2 enzymes. Examples of different PLA_2 enzymes include PLA_2 Type I, II and III.

The the present invention clearly extends to fusion polypeptides comprising one or more α-chain and β-chain polypeptides and mimotypes thereof.

TABLE 3

Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any amino acid as above	Xaa	X

TABLE 4

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva

D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib
D-valine	Dval	α-methyl-γ-aminobutyrate	Mgabu
D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
D-α-methylarginine	Dmarg	α-methylcylcopentylalanine	Mcpen
D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
D-α-methylaspartate	Dmasp	α-methylpenicillamine	Mpen
D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
D-α-methylisoleucine	Dmile	N-amino-α-methylbutyrate	Nmaabu
D-α-methylleucine	Dmleu	α-napthylalanine	Anap
D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
D-α-methylvaline	Dmval	N-cylcododecylglycine	Ncdod
D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl) glycine	Nbhm
D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl) glycine	Nbhe

D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl) glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylhistidine	Dnmhis	N-(hydroxyethyl))glycine	Nser
D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)) glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl) glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L-α-methylalanine	Mala
L-α-methylarginine	Marg	L-α-methylasparagine	Masn
L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
L-α-methylhistidine	Mhis	L-α-methylhomo phenylalanine	Mhphe
L-α-methylisoleucine	Mile	N-(2-methylthioethyl) glycine	Nmet
L-α-methylleucine	Mleu	L-α-methyllysine	Mlys

L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
L-α-methylserine	Mser	L-α-methylthreonine	Mthr
L-α-methyltryptophan	Mtrp	L-α-methyltyrosine	Mtyr
L-α-methylvaline	Mval	L-N-methylhomo phenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane	Nmbc		

As exemplified herein, the NSI of the present invention is extremely stable to pH in the range 4-12 and to high temperature, de-glycosylation or the action of denaturing agents. Similar properties exist for NAI, OSI, OMI and PTI.

The phospholipase inhibitor or its homologue, analogue, mimetic, derivative or chemical equivalent thereof as described herein is useful in a wide range of prophylactic and therapeutic applications, by virtue of the ability of the subject phospholipase inhibitor to at least inhibit Type I, II and/or III PLA_2 enzymes. The advantageous effects of the instant invention are achieved by the administration of isolated and/or recombinant phospholipase inhibitors or functional homologues, analogues, mimetics, derivatives or chemical equivalents thereof to a human or animal subject, either directly or *via* their genetic sequences. Where administration is direct, the phospholipase inhibitor may be administered alone or as a fusion molecule or in another format such as by means known to those skilled in art. Administration may be as a composition comprising at least the isolated or recombinant PLA_2 inhibitor in combination with one or more pharmaceutically acceptable carriers and/or diluents. Alternatively, where it is desirable for the subject phospholipase inhibitor to be administered by genetic means, such means include *via* an attenuated virus, a recombinant viral vector, a nucleic acid molecule, a genetic construct or by bacterial vector.

Administration means include injection, infusion, oral ingestion, suppository and nasal spray or drops amongst other routes.

Accordingly, a further aspect of the present invention contemplates a composition comprising an isolated or recombinant phospholipase inhibitor or a homologue, analogue, derivative, mimetic or chemical equivalent thereof together with one or more pharmaceutically acceptable carriers and/or diluents.

Alternatively, or in addition, the composition may comprise a mimotype of the phospholipase inhibitor.

Preferably, the composition is injected or orally administered. Where the composition comprises genetic material such as DNA or RNA, preferably it is administered as part of a viral vector, live viral vector, live bacterial vector or naked or protected nucleic acid molecule.

Conditions for which treatment might be required include any inflammatory condition, autoimmune condition, organ dysfunction or toxic poisoning which involves the action of a PLA_2 enzyme and in particular a Type I, II or III PLA_2 enzyme. More preferably, the PLA_2 inhibitor of the present invention and compositions comprising same are useful for the treatment of rheumatoid arthritis, osteoarthritis, asthma, allergy, psoriasis, multiple organ failure, acute pancreatitis, acute lung failure, septic shock, adult respiratory distress syndrome and neutralisation of allergic reactions to animals such as arachnids (eg. spiders, scorpions, mites, etc) insects (eg. wasps, bees, ants, fleas, etc), reptiles (eg. snakes, lizards, etc), amphibians (eg. toads, frogs) or aquatic animals (eg: fish, cephalopods, box jellyfish, Portuguese man-of-war jellyfish, blue-ringed octopus, etc), amongst others, or the toxic effects of toxins produced by such animals.

In an even more preferred embodiment, the PLA_2 inhibitor of the present invention and pharmaceutical compositions comprising same are useful for the treatment of inflammation associated with rheumatoid arthritis or osteoarthritis, for the treatment of snake bite,

arthropod bite, insect stings and neutralisation of the toxic effects of notexin.

By "snake bite" is meant the allergic reaction or toxic effects of any snake bite, including the allergic reaction or toxic effects of a venom produced by a snake. The present invention is particularly useful for the treatment of the toxic effects of a wide range of snake venoms, including those produced by snakes from any one or more of the families Colubridae (colubrid snakes such as species of the genera *Heterodon*, *Natrix*, *Regina*, *Clonophis*, *Thamnophis*, *Lampropeltis, Opheopdris, Coluber, Masticophis, Drymobius, Salvadora, Phyllorhyncus, Elaphe, Hydrodunastes, Ptyas, Calamaria, Lycodon, Mehelya, Boaedon, Farancia, Fordonia, Erpeton,* amongst others), Elapidae (cobras such as species of the genera *Ophiophagus, Naja, Oxyuranus, Pseudohaje, Walterinnesia, Aspidelaps, Boulengerina, Dendroaspis, Bungaris, Calliophis, Maticora, Micurus, Micruroides, Acanthophis, Notechis* and *Australaps*, amongst others), Hydrophiidae (sea snakes such as species of genera *Laticauda, Aipysurus*, *Hydrophis* and *Enhydrina*, amongst others), Viperidae (vipers, such as species of the genera *Viptera, Echis, Cerastes, Bitis, Atractaspis* and *Causus,* amongst others) and Crotalidae(pit vipers such as species of genera *Crotalis, Sistrurus, Bothrops, Trimeresurus, Lachesus* and *Agkistrodon,* amongst others).

Even more preferably, the PLA_2 inhibitor of the present invention or its homologue, analogue, mimetic, derivative or chemical equivalent thereof and compositions comprising same are useful in treatment of snakes bite, wherein the snakes are from the family Viperidae, such as *Viptera* spp. and *Bitis* spp., in particular, *V. russelli, A. bilineatus* and *B. alternatus* ; family Crotalidae, such as moccasin snakes and vipers (*Agkistrodon* spp.) and rattlesnakes (*Crotalus* spp.), in particular *Crotalus atrox*; or the family Elapidae, such as but not limited to King cobra *(Ophiohagus hannah);* True cobras *(Naja* spp); Asian or Indian cobra *(N. naja);* Egyptian cobra *(N. haje);* Spitting cobra *(N. nigricolli);* Black-lipped cobra *(N. malenoleuca);* Cape cobra *(N. nivea);* Gold's tree cobra *(Pseudohaje goldii);* Desert black snakes *(Walterinnesia* spp); Shield-nose snakes *(Aspidelaps* spp); Water cobras or water snakes *(Boulengerina* spp); Black mamba *(Dendroaspis polylepis);* Mamba *(D. angusticeps);* Kraits snake *(Bungarus* spp); Oriential coral snakes *(Calliophis* spp); Long-glanded coral snakes *(Maticora* spp); American coral snakes *(Micurus* spp); Southern coral snake *(M.*

frontalis); Eastern coral snake or Harlequin snake *(M. fulvius);* Western coral snake *(Micruroides* spp); Arizona coral snake *(M. euryxanthus);* Death adder *(Acanthophis antarcticus);* Australian tiger snakes *(Notechis* spp); and Australian copperhead *(Australaps* spp), amongst others.

By "arthropod bite" is meant an allergic reaction or toxic effects of any arthropod bite, including the allergic reaction or toxic effects of a venom produced by said arthropod. The arthropod may be a spider, in particular a venomous spider such as a funnel web spider, red-back spider, amongst others or a scorpion, pseudoscorpion, mite, wasp, bee or ant, amongst others.

By "insect sting" is meant an allergic reaction or toxic effects of any insect sting, including the allergic reaction or toxic effects of a venom produced by said insect. The insect may be a wasp, bee or ant, amongst others.

The pharmaceutical compositions of the present invention may also contain other active molecules or ingredients such as antibiotics to prevent infection at a wound site induced by the animal causing the sting or bite (e.g. the arachnid, insect, reptile, amphibian or aquatic animal) and/or antigen molecules, to promote protective immunity against one or more antigens present in the venom produced by said animal.

The active ingredient(s) of composition is/are contemplated to exhibit excellent PLA_2 inhibitory activity in animals and humans when administered. Variations in dosage administration occur depending, for example, on activity of the phospholipase enzyme required to be inhibited and I_{50} inhibitor, intended purpose of administration, such as whether for use as an anti-inflammatory agent or as an anti-toxin and particularly in the case of toxic poisoning and the delay between the onset of symptoms and the commencement of treatment. Dosage regimen may be adjusted without undue experimentation by those skilled in the art to provide the optimum therapeutic response. For example, several divided doses may be administered in one or more of hourly, daily, weekly or monthly or in other suitable time intervals or the dose may be proportionally reduced as indicated by the exigencies of

situation.

For toxicological applications, those skilled in art will be aware that the optimum dosage required should be calculated based upon LD_{50} value of the particular toxin being neutralised and such calculations are well-within the capacity of such persons. For example, successful neutralisation of notexin is observed in mice administered with between 1-fold and 4-fold the toxic dose of notexin.

The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount of active ingredient; a powder or granules; a solution or a suspension in an aqueous or non-aqueous liquid. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. inert diluent), preservative disintegrant (e.g. sodium starch glycolate, cross-linked polyvinyl pyrrolidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of powdered compound moistened with an inert liquid diluent.

Tablets or powders or granules may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile.

Additionally, sweeteners or dietary formulae may be included to improve their palatability to a specific animal subject. Optionally, such solid compositions are provided with an enteric coating, to provide release in parts of gut or than stomach.

The active compounds may also be administered in dispersions prepared in glycerol, liquid polyethylene glycols, and/or mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

Pharmaceutical forms suitable for parenteral administration include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by use of a coating such as lecithin, by the maintenance of the required particle size in the case of a dispersion and by use of surfactants. The prevention of action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimersal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by their use in compositions of agents delaying absorption, for example.

Sterile injectable solutions are prepared by incorporating active compounds in the required amount in appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilisation. Generally, dispersions are prepared by incorporating various sterilised active ingredient(s) into a sterile vehicle which contains the basic dispersion medium and required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of

preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

The compositions may also be delivered by a live delivery system such as using a bacterial expression system to express the PLA_2 inhibitor in bacteria which can be incorporated into gut flora. Alternatively, a viral expression system can be employed. In this regard, one form of viral expression is administration of a live vector generally by spray, feed or water where an infecting effective amount of live vector (e.g. virus or bacterium) is provided to the animal. Another form of viral expression system is a non-replicating virus vector which is capable of infecting a cell but not replicating therein. The non-replicating viral vector provides a means of introducing to the human or animal subject genetic material for transient expression therein to produce the PLA_2 inhibitory protein. The mode of administering such a vector is the same as a live viral vector.

The carriers, excipients and/or diluents utilised in the compositions of the present invention should be acceptable for human or veterinary applications. Such carriers, excipients and/or diluents are well-known to those skilled in the art. Suitable carriers and/or diluents include any and all solvents, dispersion media, aqueous solutions, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. Except insofar as any conventional medium or agent is incompatible with the active ingredient, use thereof in the composition is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The compositions of this invention may include other agents conventional in the art. For example, compositions suitable for oral administration may include such further agents as dietary formulae, binders, sweeteners, thickeners, flavouring agents disintegrating agents, coating agents, preservatives, lubricants and/or time delay agents. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, xanthan gum, bentonite, alginic acid or agar. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry,

orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or ir esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

A further aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides which encodes or is complementary to a sequence which encodes a phospholipase inhibitor or a homologue or derivative of said phospholipase inhibitor.

The present invention extends to derivatives, homologues and analogues of the subject nucleic acid molecule.

The origin of the isolated nucleic acid molecule is not essential to the performance of the present invention, the only requirement being that the expression product of the subject nucleic acid molecule is at least capable of inhibiting a phospholipase enzyme such as PLA_2, in particular a Type I, II or III PLA_2 enzyme, and more particularly, two or more of Type I, II and/or III PLA_2 enzymes.

The person of ordinary skill in the relevant art would, without any undue experimentation, be capable of determining an appropriate source of the subject nucleic acid molecule and obtaining same in order to perform the embodiments described herein.

Preferably, the nucleic acid molecule is originally derived from a species of animal which produces a toxin which exerts its effect *via* activity of a PLA_2 enzyme. In a particularly preferred embodiment, nucleic acid molecule of the present invention is derived from a venomous animal such as a snake. In a particularly preferred embodiment, the isolated nucleic acid molecule is derived from *Notechis* spp. such as, but not limited to *N. scutatus* or *N. ater* or an *Oxyuranus* spp. Such as but not limited to *O. scutellatus* or *O. microlepidotus* or a *Pseudonaja* species such as but not limited to *P. textilis*. Ultimately, nucleic acid

molecule may be derived or maintained in an animal cell line, insect cells, eukaryotic cells or bacterial cells.

This aspect of the present invention clearly extends to any isolated gene which encodes a PLA_2 inhibitor or a peptide or polypeptide subunit thereof or a homologue or derivative of said inhibitor including a fusion or hybrid molecule encoding a fusion or hybrid PLA_2 inhibitor.

Reference herein to a "gene" is to be taken in its broadest context and includes:

(i) a classical genomic gene consisting of transcriptional and/or translational regulatory sequences and/or a coding region and/or non-translated sequences (i.e. introns, 5′- and 3′- untranslated sequences);

(ii) mRNA or cDNA corresponding to the coding regions (i.e. exons) optionally comprising 5′- or 3′-untranslated sequences of the gene; or

(iii) an amplified DNA fragment or other recombinant nucleic acid molecule produced *in vitro* and comprising all or a part of the coding region and/or 5′- or 3′-untranslated sequences of the gene.

The term "gene" is also used to describe synthetic or fusion molecules encoding all or part of a functional product. A functional product is one which comprises a sequence of nucleotides or is complementary to a sequence of nucleotides which encodes a functional PLA_2 inhibitor and in particular NSI, NAI, OSI, OMI and/or PTI or a homologue or derivative thereof.

Genes of the present invention may be derived from a naturally-occurring PLA_2 inhibitor-encoding gene by standard recombinant techniques. Generally, a PLA_2 inhibitor-encoding gene may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or additions. Nucleotide insertional derivatives of the PLA_2 inhibitor encoding gene of the present invention include 5′ and 3′ terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in the nucleotide sequence although random insertion is also possible with suitable screening of the resulting

product. Deletional variants are characterised by the removal of one or more nucleotides from the sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide inserted in its place. Such a substitution may be "silent" in that the substitution does not change the amino acid defined by the codon. Alternatively, substitutions are designed to alter one amino acid for another similar acting amino acid, or amino acid of like charge, polarity or hydrophobicity.

Accordingly, the present invention extends to homologues, analogues and derivatives of a gene which encodes a phospholipase inhibitor as herein described.

For the present purpose, "homologues" of a gene as hereinbefore defined or of a nucleotide sequence shall be taken to refer to an isolated nucleic acid molecule which is substantially the same as the nucleic acid molecule of the present invention or its complementary nucleotide sequence, notwithstanding the occurrence within said sequence of one or more nucleotide substitutions, insertions, deletions, or rearrangements.

"Analogues" of a gene as hereinbefore defined or of a nucleotide sequence set forth herein shall be taken to refer to an isolated nucleic acid molecule which is substantially the same as a nucleic acid molecule of the present invention or its complementary nucleotide sequence, notwithstanding occurrence of any non-nucleotide constituents not normally present in said isolated nucleic acid molecule, for example carbohydrates, radiochemicals including radionucleotides, reporter molecules such as, but not limited to DIG, alkaline phosphatase or horseradish peroxidase, amongst others.

"Derivatives" of a gene as hereinbefore defined or of a nucleotide sequence set forth herein shall be taken to refer to any isolated nucleic acid molecule which contains significant sequence similarity to said sequence or a part thereof. Generally, the nucleotide sequence of the present invention may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or insertions. Nucleotide insertional derivatives of the nucleotide sequence of the present invention include 5´ and 3´ terminal fusions as well as intra-sequence insertions of single or multiple nucleotides or nucleotide analogues.

Insertional nucleotide sequence variants are those in which one or more nucleotides or nucleotide analogues are introduced into a predetermined site in the nucleotide sequence, although random insertion is also possible with suitable screening of the resulting product being performed. Deletional variants are characterised by the removal of one or more nucleotides from the nucleotide sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide or nucleotide analogue inserted in its place.

Particularly preferred derivatives are those which encode polypeptides capable of forming an interactive site with a phospholipase enzyme, for example by reproducing or imitating the NSI:PLA_2 interactive site or alternatively, derivatives which encode polypeptides capable of at least binding to the active site of a phospholipase enzyme.

In a further alternative embodiment, the present invention provides an isolated nucleic acid molecule having a sequence of nucleotides or complementary sequence of nucleotides comprising one or more of SEQ ID NOs. 5-8, 13-16, 39-42, 47-50, 54-56, 60-62, 67-70, 75-78, 82 to 84 or 88-90 or a nucleotide sequence having at least 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to any one or more of said sequences under low stringency conditions at 42°C.

Preferred sequence similarities or identifies include at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90% or above such as at least about 93%, 95% or 97-100%.

The present invention clearly extends to any partial or full-length cDNA and genomic clone equivalents of SEQ ID NOs. 5-8, 13-16, 39-42, 47-50, 54-56, 60-62, 67-70, 75-78, 82-84 or 88-90 and any homologues or derivatives thereof, in particular those homologues, analogues or derivatives which are obtainable using the genetic sequences herein provided.

In an alternative embodiment, the present invention extends to any isolated nucleic acid molecule which is at least capable of encoding an amino acid sequence set forth in any one of

SEQ ID NOS: 1-4, 9-12, 17-38, 43-46, 51-53, 57-59, 63-66, 71-74, 79-81 or 85-87 which is at least capable of encoding the α-chain and/or β-chain polypeptide subunits of a phospholipase inhibitor or a homologue, analogue, derivative, mimetic or chemical equivalent thereof.

The present invention further encompasses any isolated nucleic acid molecules which comprise at least a part of a PLA_2 inhibitor-encoding gene sequence as herein described. Preferably, such parts comprise at least about 10 contiguous nucleotides in length, more preferably at least about 15 contiguous nucleotides in length, even more preferably at least about 30 contiguous nucleotides in length and still even more preferably at least about 50 contiguous nucleotides in length, of SEQ ID NOS: 5-8, 13-16, 39-42, 47-50, 54-56, 60-62, 67-70, 75-78, 82-84 or 88-90 or of a sequence capable of hybridizing to these sequences under low stringency conditions at 42°C.

In a particularly preferred embodiment, the present invention provides a nucleic acid molecule which encodes an α-chain polypeptide of a PLA_2 inhibitor protein or a homologue or derivative thereof which nucleotide sequence has at least about 75% similarity to one or more of the nucleotide sequences set forth in SEQ ID NOs. 5-7, 13-15, 39-41, 47-49, 54-55, 60, 61, 67, 68, 75, 76, 82 or 88 or a nucleotide sequence capable of hybridizing under low stringency conditions at 42°C to one or more of said sequence.

More preferably, percentage similarity is at least about 90%, including at least 91% or 93% or 95% or 100%.

In an alternative embodiment, the present invention contemplates an isolated nucleic acid molecule which encodes a β-chain polypeptide or a PLA_2 inhibitor protein or a homologue or derivative thereof which has at least about 75% similarity to any one of the nucleotide sequences set forth in SEQ ID NOs:8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89 or 90 or a nucleotide sequence capable of hybridising under at least low stringency conditions at 42°C to one of said sequences.

In a preferred embodiment of invention, the hybridisation stringency is at least medium stringency. More preferably, the hybridisation stringency is at least a high stringency.

In a particularly preferred embodiment, the nucleic acid molecule of the present invention is further characterised as a PLA_2 inhibitor encoding gene.

Yet another aspect of the present invention provides an nucleic acid molecule encoding hybrid PLA_2 inhibitors.

Accordingly, the present invention provides a nucleic acid molecule encoding a PLA_2 inhibitor having the structure:

$$\alpha_m\beta_n$$

wherein

α is an α-chain of a PLA_2 inhibitor;

β is a β-chain of a PLA_2 inhibitor; m is an integer from 0 to 10;

n is an integer from 0 to 10 with proviso that if m and n are not 0, then m>n and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α comprises an amino acid sequence selected from SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 and 85 or an amino acid sequence having at least about 40% similarity to one or more of said sequences and β comprises an amino acid sequence selected from SEQ ID NOs: 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 and 87 or an amino acid sequence having at least about 40% similarity to one or more of said sequences.

In another embodiment, the present invention is directed to a nucleic acid molecule encoding a PLA_2 inhibitor having the structure:

$$\alpha_m\beta_n$$

wherein

α is an α-chain of a PLA_2 inhibitor;

β is a β-chain of a PLA_2 inhibitor; m is an integer from 0 to 10;

n is an integer from 0 to 10 with proviso that if m and n are not 0, then m>n and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α is encoded by an nucleotide sequence selected from SEQ ID NOs. 5-7, 13-15, 39-41, 47-49, 54, 55, 60, 61, 67, 68, 75, 76, 82 and 88 or a nucleotide sequence having at least about 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to one or more of said sequences under low stringency conditions at 42°C and β is encoded by a nucleotide sequence selected from SEQ ID NOs: 8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89, 90 or a nucleotide sequence capable of hybridizing to one or more of said sequences under low stringency conditions at 42°C.

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions. In general, washing is carried out $T_m = 69.3 + 0.41$ (G+C)% (Marmur and Doty, 1962). However, the T_m of a duplex DNA decreases by 1°C with every increase of 1% in number of mismatch base pairs (Bonner and Laskey, 1974).

The functional genetic sequences of the present invention are useful for expression of the PLA_2 inhibitor in cells. Non-functional genetic sequences which do not express a functional PLA_2 inhibitor are at least useful as, for example, genetic probes, primer sequences, antisense or sense molecules or in the generation of immunologically interactive recombinant molecules.

In a particularly preferred embodiment, the PLA_2 inhibitor-encoding genes and homologues or derivatives thereof are employed to identify and isolate similar genes from other sources. The present invention extends to all such applications.

Related PLA_2 inhibitor-encoding genes are isolated by contacting genomic DNA, or mRNA, or cDNA, or an amplified gene product or a part, fragment or a source thereof, with a hybridisation effective amount of a probe and then detecting the hybridisation. The related genetic sequence may be in a recombinant form, in a virus particle, bacteriophage particle, yeast cell, animal cell or a plant cell. In addition, the related genetic sequence may be bound to a support matrix or membrane comprising, for example, nylon, nitrocellulose, polyacrylamide or agarose, amongst others. The probe is generally labelled with a reporter molecule capable of giving an identifiable signal (e.g. a radioisotope such as ^{32}P or ^{35}S or a biotinylated molecule).

An alternative method contemplated by the present invention involves hybridising a nucleic acid primer molecule of at least 10 nucleotides in length derived from the nucleotide sequence herein described to a nucleic acid "template molecule", said template molecule herein defined as a related PLA_2 inhibitor-encoding gene or a functional part thereof, or its complementary sequence. Specific nucleic acid molecule copies of the template molecule are amplified enzymatically in a polymerase chain reaction, a technique that is well known to those skilled in the art and which is described by McPherson *et al.* (1991).

Preferably, the nucleic acid primer molecule or the molecule effective in hybridisation is contained in an aqueous mixture of other nucleic acid primer molecules. More preferably, the nucleic acid primer molecule is in a substantially pure form. The nucleic acid template molecule may be in a recombinant form, in a virus particle, bacterial cell, bacteriophage particle, yeast cell, animal cell, or a plant cell. For production of recombinant protein in isolated cells, the nucleic acid molecule of the present invention is placed, in the sense orientation, in operable connection with a suitable promoter sequence and introduced into a suitable expression system, for example a bacterial, yeast, baculovirus, plant, animal or other expression system.

Accordingly, a further aspect of the present invention provides a genetic construct comprising an isolated nucleic acid molecule which comprises a sequence of nucleotides which corresponds to, or is complementary to a phospholipase inhibitor-encoding gene or a homologue or derivative thereof.

According to this embodiment, the coding region of a phospholipase inhibitor encoding gene may be placed in operable connection with a promoter sequence such that a gene product is capable of being expressed under the control of said promoter sequence.

Optionally, said genetic construct further comprises a terminator sequence.

In present context, the term "in operable connection with" is used to indicate that expression of the isolated nucleotide sequence is under the control of the promoter sequence with which it is connected.

The term "terminator" refers to a DNA sequence at the end of a transcriptional unit which signals termination of transcription. Terminators are 3′-non-translated DNA sequences containing a polyadenylation signal, which facilitates the addition of polyadenylate sequences to the 3′-end of a primary transcript. Terminators active in plant cells are known and described in the literature. They may be isolated from bacteria, fungi, viruses, animals and/or plants.

Examples of terminators particularly suitable for use in the genetic constructs of the present invention include SV40 polyadenylation signal, amongst others.

Reference herein to a "promoter" is to be taken in its broadest context and includes transcriptional regulatory sequences of a classical genomic gene, including a TATA box which is required for accurate transcription initiation in eukaryotic cells, with or without a CCAAT box sequence and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers). For expression in prokaryotic cells, such as bacteria, the promoter

should at least contain the -35 box and -10 box sequences.

A promoter is usually, but not necessarily, positioned upstream or 5′, of the phospholipase inhibitor encoding gene, the expression of which it regulates. Furthermore, the regulatory elements comprising a promoter are usually positioned within 2 kb of start site of transcription of gene.

In the present context, the term "promoter" is also used to describe a synthetic or fusion molecule, or a derivative which confers, activates or enhances expression of an isolated nucleic acid molecule, in a cell, such as a plant, animal, insect, fungal, yeast or bacterial cell. Preferred promoters may contain additional copies of one or more specific regulatory elements, to further enhance expression of a nucleic acid molecule which expression it regulates and/or to alter the spatial expression and/or temporal expression of same. For example, regulatory elements which confer copper inducibility may be placed adjacent to a heterologous promoter sequence driving expression of a nucleic acid molecule, thereby conferring copper inducibility on the expression of said molecule.

Placing an isolated nucleic acid molecule under the regulatory control of a promoter sequence means positioning said molecule such that expression is controlled by the promoter sequence. Promoters are generally positioned 5′ (upstream) to genes that they control. In construction of heterologous promoter/structural gene combinations, it is generally preferred to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it controls in its natural setting, i.e., the gene from which the promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of promoter function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by positioning of the element in its natural setting, i.e., the genes from which it is derived. Again, as is known in the art, some variation in this distance can also occur.

Examples of promoters suitable for use in genetic constructs of the present invention include

viral, fungal, bacterial, animal and plant derived promoters capable of functioning in plant, animal, insect, fungal, yeast or bacterial cells. The promoter may regulate the expression of the nucleic acid molecule constitutively, differentially with respect to the tissue in which expression occurs or, with respect to developmental stage at which expression occurs, or inducibly such as in response to external stimuli including physiological stresses, plant pathogens, metal ions, effector molecules, amongst others.

Preferably, the promoter is capable of regulating expression of a nucleic acid molecule in a yeast or bacterial cell.

Examples of preferred promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, *lac* promoter, *tac* promoter, SV40 early promoter and the like.

The genetic construct contemplated herein is introduced into a suitable expression system for a time and under conditions sufficient for expression of the PLA_2 inhibitor gene to occur.

Accordingly, a further aspect of invention contemplates a recombinant phospholipase inhibitor, produced by expressing a nucleic acid molecule described herein in a suitable host cell. The present invention extends also to a synthetic peptide fragment of said recombinant gene product.

It is to be understood that the recombinant and isolated PLA_2 inhibitors described herein include homopolymers and heteropolymers of an α-chain and/or β-chain of a PLA_2 inhibitor. The α- and β- chains may be produced independently as recombinant proteins and reassociated into active molecules. They may occur *in vitro* or *in vivo*. For example, the two chains could be expression as separate proteins by the same recombinant cell (bacteria, yeast, insect or mammalian) using several different genetic constructs. A construct could be produced where the expressed protein consists of α- and β- chains joined by a linker peptide in a manner analogous to the structure of single chain Fv recombinant antibody fragments. The two such molecules would then self-associate to form the appropriate structure.

With particular regard to recombinant polypeptides, those skilled in the art will be aware of methods which may be employed to produce such multimeric proteins, for example, by co-expression of polypeptide subunits on separate genetic constructs or the same genetic construct, in a suitable cellular host. Alternatively, α- and β-chain subunits of a PLA_2 inhibitor may be expressed as a fusion polypeptide.

The present invention clearly extends to the recombinant production of polypeptide mimotypes of NSI, NAI, OSI, OMI and PTI or their homologues.

Still further aspect of the present invention provides a method of isolating a PLA_2 inhibitor from snake blood, serum or other blood component, said method comprising steps of:

(i) preparing a serum sample from clotted blot; and/or

(ii) subjecting blood serum to ion-exchange chromatography.

The ion exchange chromatography may be performed using an anion exchanger or cation exchanger.

The composition of buffers used for each of the steps of the subject method may be determined by the person skilled in the art, without undue experimentation, the only requirement of such buffer compositions being that they are suitable for the maintenance of activity of the PLA_2 inhibitor being purified under chromatographic procedure employed.

The buffer compositions may additionally include at least one, preferably two and more preferably three protease inhibitors to prevent proteolysis of enzyme during the purification procedure, in particular a trypsin inhibitor.

Preferably, the purification is carried out as described in Example 1, for the purification of the *Notechis scutatus* PLA_2 inhibitor NSI. A simple procedure is also effective for NAI, OSI, OMI and PTI.

The present inventors compared amino acid and corresponding nucleotide sequences for NSI, NAI, OSI, OMI and PTI and determined the consensus sequences for the alpha and beta

chains. The present invention extends, therefore, to isolated polypeptides and corresponding genetic sequences which are encompassed by these consensus sequences.

Accordingly, another aspect of the present invention provides an isolated polypeptide capable of inhibiting two or more of PLA_2 Type I, II and/or III wherein said polypeptide has an alpha chain comprising the following amino acid sequence:

Xaa Ser Cys Glu Xaa Cys Xaa Asn Xaa Gly Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Glu Cys Ala Ser Xaa Glu Asp Gln Cys Gly Thr Val Leu Xaa Glu Xaa Ser Xaa Ala Pro Ile Ser Xaa Arg Xaa Ile His Arg Xaa Cys Phe Ser Ser Ser Xaa Cys Lys Leu Glu Xaa Phe Asp Ile Asn Ile Gly His Asp Ser Xaa Xaa Arg Gly Arg Ile His Cys Cys Xaa Glu Xaa Xaa Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn Gly Tyr His Cys Pro Gly Ile Xaa Gly Xaa Phe Ser Val Asp Ser Ser Glu His Glu Ala Ile Cys Arg Gly Xaa Glu Thr Lys Cys Ile Xaa Xaa Ala Gly Phe Arg Xaa Glu Arg Xaa Xaa Xaa Asp Xaa Xaa Tyr Asn Ile Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Xaa Leu Xaa Asn Arg Thr His Xaa Xaa Xaa Xaa Asn Xaa Leu Ile Xaa Xaa Glu Cys Thr Xaa Ala Xaa Lys Xaa Xaa Pro Ser Glu.

Preferably, the isolated polypeptide is encoded by the following nucleotide sequence:

CNCTCATGTGAAANTTGTCNCAATTTNGGAANAGNNTGNNANNNTGNNNNGNCA
NNGGAATGTGCNTCTNCAGAAGATCAATGTGGCACNGTGTTGNTGGAGNTTTCA
NCNGCACCTATTTCCNNCCGANCCATTCANAGGAANTGTTTCTCATCCAGCNTCT
GCAAACTNGAACNNTTTGATATAAATATTGGACATGATTCCTNTNTNAGAGGAA
GAATCCACTGTTGTNATGAAGNAANGTGNGAAGCACAGCAATTTCCTGGACTGC
CCCTCTCCTTTCCAAATGGATANCACTGCCCTGGNATNNTTGGTNNATTCTCAGT
GGACAGNTCTGAACATGAAGCTATTTGCAGAGGAANNGANACCAAATGCATTAA
NNTTGCGGGATTCAGAANNGAAAGANNTNNNNNAGACATNGNTTATAATATCAA
AGGTTGCACTTCTTCTTGTCCAGAACTGANGTTGANNNATAG

or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C.

Yet another aspect of the present invention provides an isolated nucleic acid molecule encoding an alpha chain of a PLA_2 inhibitor said nucleic acid molecule comprising the nucleotide sequence:

CNCTCATGTGAAANTTGTCNCAATTTNGGAANAGNNTGNNANNNTGNNNNGNCA
NNGGAATGTGCNTCTNCAGAAGATCAATGTGGCACNGTGTTGNTGGAGNTTTCA
NCNGCACCTATTTCCNNCCGANCCATTCANAGGAANTGTTTCTCATCCAGCNTCT
GCAAACTNGAACNNTTTGATATAAATATTGGACATGATTCCTNTNTNAGAGGAA
GAATCCACTGTTGTNATGAAGNAANGTGNGAAGCACAGCAATTTCCTGGACTGC
CCCTCTCCTTTCCAAATGGATANCACTGCCCTGGNATNNTTGGTNNATTCTCAGT
GGACAGNTCTGAACATGAAGCTATTTGCAGAGGAANNGANACCAAATGCATTAA
NNTTGCGGGATTCAGAANNGAAAGANNTNNNNNAGACATNGNTTATAATATCAA
AGGTTGCACTTCTTCTTGTCCAGAACTGANGTTGANNNATAG

or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C.

Still a further aspect of the present invention is directed to an isolated polypeptide capable of inhibiting two or more of PLA_2 Type I, II and/or III wherein said polypeptide has a beta chain comprising the following amino acid sequence:

Leu Glu Cys Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Asn Xaa Xaa Thr Lys Thr
Cys Asp Ala Asn Gln Asp Xaa Cys Val Thr Xaa Gln Thr Glu Val Ile Arg Ala Pro Val Ser
Leu Xaa Xaa Ile Ser Lys Ser Cys Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Xaa Glu Thr
Ser Pro His Asn Glu Leu Thr Val Lys Thr Lys Arg Thr Cys Cys Thr Gly Glu Glu Cys Lys
Thr Leu Pro Pro Pro Val Leu Gly Xaa Lys Val Xaa Pro Pro Asn Gly Leu Gln Cys Pro Gly
Cys Xaa Gly Leu Ser Ser Lys Glu Cys Thr Glu His Xaa Val Ser Cys Arg Gly Ser Glu Asn
Gln Cys Leu Ser Xaa Ile Gly Lys Glu Phe Gly Xaa Phe Phe Arg Ala Leu Ser Tyr Lys Gly
Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Xaa Phe Trp Asn Val Leu Glu Xaa Val
Glu Val Asp Phe Lys Cys Xaa Pro Ala Leu Pro Lys Ser Ser Gln.

Preferably, this isolated polypeptide is encoded by the following nucleotide sequence:

CTTGAGTGNGANNTTTGTNTNNNGCNNGNCCNGNAATGTNNNAACNNCGGACG
AAAACCTGTGANGCTAATCAAGATNCTTGTGTTACNTNTCAAACTGAAGTGATA
AGAGCCCCTGTGTCCCTCNCTTTNATNTCAAAATCCTGTGGTACTTCTGACACTT
GCCATCTTAACTACNTGGAGACGAGTCCACATAATGAACTAACNGTGAAGACCA
AAAGAACCTGCTGTACTGGGGAGGAATGTAAAACTCTGCCACCGCCTGTGCTTG
GANACAAAGTCANCCCACCCAACGGACTTCAGTGTCCTGGATGCNTTGGATTGT
CCTCAAAAGAATGCACTGAACACCNGGTTTCCTGCCGGGGATCTGAAAACCAGT
GNNTGTCNNTAATTGGGAANGAATTTGGCNTTTTCTTCAGAGCATTGTCTTATAA
AGGATGTGCTACGGAGAGTCTGTGCACTNTATTTGAGAAGANGTTCTGGAATGT
TTTAGAGGANGTTGAAGTAGACTTCAAATGCNCNCCNGCCCTCCCAAAGTCTTCC
CAGNNN

or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C.

Even yet a further aspect of the present invention provides an isolated nucleic acid molecule encoding a beta chain of a PLA_2 inhibitor said nucleic acid comprising the nucleotide sequence:

CTTGAGTGNGANNTTTGTNTNNNGCNNGNCCNGNAATGTNNNAACNNCGGACG
AAAACCTGTGANGCTAATCAAGATNCTTGTGTTACNTNTCAAACTGAAGTGATA
AGAGCCCCTGTGTCCCTCNCTTTNATNTCAAAATCCTGTGGTACTTCTGACACTT
GCCATCTTAACTACNTGGAGACGAGTCCACATAATGAACTAACNGTGAAGACCA
AAAGAACCTGCTGTACTGGGGAGGAATGTAAAACTCTGCCACCGCCTGTGCTTG
GANACAAAGTCANCCCACCCAACGGACTTCAGTGTCCTGGATGCNTTGGATTGT
CCTCAAAAGAATGCACTGAACACCNGGTTTCCTGCCGGGGATCTGAAAACCAGT
GNNTGTCNNTAATTGGGAANGAATTTGGCNTTTTCTTCAGAGCATTGTCTTATAA
AGGATGTGCTACGGAGAGTCTGTGCACTNTATTTGAGAAGANGTTCTGGAATGT
TTTAGAGGANGTTGAAGTAGACTTCAAATGCNCNCCNGCCCTCCCAAAGTCTTCC

CAGNNN

or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C.

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The present invention is further described with reference to the following non-limiting Examples.

EXAMPLE 1

Purification of Phospholipase A_2 Inhibitor from Snake Blood

Unless otherwise stated all experiments were performed at room temperature.

Tiger snake (*N. scutatus*) blood was collected and allowed to clot. The blood was then centrifuged at 1,500 x g for 15 minutes. The serum was then collected and stored at -20°C. Serum was extensively dialysed against 0.01M ammonium acetate (NH_4OAc), pH 7.0. The *N. scutatus* phospholipase A_2 inhibitor (NSI) was purified using anion exchange chromatography.

Dialysed serum was loaded (up to 15mL at ~20mg/mL) onto a DEAE-Sephacel column (20 x 1.5cm) that has been equilibrated with 0.01M NH_4OAc, pH 7.0 at a flow rate of 0.5mL/min. A step gradient was then developed as follows: 0.1 NH_4OAc, 0.25M NH_4OAc, 0.5 NH_4OAc and 1.0M NH_4OAc (all pH 7.0). The eluent was monitored at 280nm with an Isco type 11 detector. The concentration of NH_4OAc was not increased until the preceding peak has fully eluted. NSI eluted the 0.5M NH_4OAc step. The procedure was performed at 4°C.

The sample was then concentrated by lyophilisation and then resuspended in water and stored at -20°C. Alternatively, if a large volume was collected (>15mL), the sample was concentrated using an Amicon ultrafiltration device fitted with a YM 10 membrane. This semi-purified preparation (SPP) of NSI was approximately 90-95% pure.

NSI can be purified to >98% purity using cation exchange chromatography. A Mono-S HR 5/5 column was equilibrated with 10mM sodium acetate pH 5.5. The SPP NSI fraction was applied and a gradient developed with 430mM sodium acetate pH 5.5 as follows:

(i) 0-3 minutes 0%;

(ii) 3-8 minutes 0-20%;

(iii) 8-20 minutes 20-40%;

(iv) 20-25 minutes 40-60%; and

(v) 25-30 minutes 60-100%.

NSI eluted in the 20-40% section of the gradient (Figure 1a and 1b).

EXAMPLE 2

Phospholipase A_2 assays and inhibition of Phospholipase A_2 activity by NSI

Phospholipase A_2 activity was assigned using a modification of the method of Radvanyi *et al.* (1989). This assay is based on the ability to measure the fluorescence emitted by an artificial substrate after it has been cleaved by a PLA_2 enzyme. The level of fluorescence is proportional to the amount of cleaved substrate which is in turn proportional to enzymatic activity. The phospholipid substrate, labelled in the sn-2 position with 10-pyrenyldecanoic acid, forms micelles upon addition to the reaction medium. The fluorescence of the substrate is quenched by pyrene-pyrene interactions. Upon hydrolysis the free 10-pyrenyldecanoic acids are absorbed by bovine serum albumin (BSA) and the fluorescence emitted is measured. The artificial substrate 1-hexadecanoyl-2-(1-predecanoyl)-sn-glycero-3-phosphocholine (10pPC [Molecular Probes, Inc.]) was dissolved (1mg) in 5.87mL 95% v/v ethanol to yield a 0.2M stock solution. Aliquots of 200μL were stored at -20 C for up to 3 months.

To 1mL of assay buffer (50mM Tris [hydroxymethyl]methylamine-HCl[Tris]), pH7.5, 100mM NaCl, and 1mM ethylenediaminetetra-acetic acid [EDTA]) the following were added sequentially; 16μL of a 1:0.6 (v/v) mixture of 10% (w/v) BSA and 1M $CaCl_2$ (0.1% w/v and
2μM final concentration, respectively), 10μL 10pPC stock solution, injected quickly to facilitate micellular formation. To this, 35μL of a test sample, PLA_2 source plus SPP or water, or saline/BSA, was added. This solution was mixed well with shaking. The substrate was excited at 345nm and the fluorescent spectrometer for 4 minutes.

EXAMPLE 3

Inhibition of a variety of snake venom phospholipase A_2 activities by partially-purified *N. scutatus* phospholipase A_2 inhibitor

Using the SPP fraction prepared according to Example 1, inhibition of the phospholipase A_2 activities of a wide range of snake venoms was tested. The venoms tested were; *N.scutatus* (homologous venom), *P.textilis*, *N.melanoleuca* (family; Elapidae), *V.russelli* (family; Viperidae), *A. bilineatus*, *B.alternatus* and *C.atrox* (family; Viperidae, subfamily; Crotalinae.

First, an appropriate dilution of venom was established for use in the assay described in Example 2. The criteria required a substantial change in fluorescent intensity over a relatively short period of time. Venoms were diluted to achieve a phospholipase A_2 enzyme activity sufficient to produce a change of 250 fluorescent intensity units over 70-80 seconds in the absence of any inhibitor. As such all venoms showed similar PLA_2 activity in the assay. A 1mg/mL solution of each venom was made up fresh when it was to be tested. Dilutions (of the 1mg/mL solution) used in the assay are as follows; *N.scutatus* 1/200, *P.textilis* 1/20, *N.melanoleuca* 1/150, *V.russelli* 1/15, *A.bilineatus* 1/20, *B.alternatus* 1/10 and *C.atrox* 1/10.

The SPP fraction was also diluted prior to testing against each venom. The dilutions were; 1/2, 1/8, 1/12, 1/50, 1/100 and 1/200 of a 1.11mg/mL solution.

The SPP dilutions were incubated with each diluted venom sample in the ratio 2.5:1 (v/v) before assaying phospholipase A_2 enzyme activity. Three assays were performed for each dilution of SPP on each day. Control samples were assayed both before and after each dilution was tested. The control consisted of venom plus water in the same ratio as the SPP:venom. Three batches were assayed daily with separate controls for each batch. All samples were prepared at the same time and then selected randomly for testing. All samples being tested were kept on ice. Samples not used immediately were stored at -20°C.

Results are expressed as percentage inhibition compared to control values. (Figure 2a and 2b). As shown in Figures 2a and 2b, the SPP fraction of *N.scutatus* phospholipase A_2

inhibitor was most effective at inhibiting the activities of *N.scutatus* snake venom phospholipase A_2, with at least 80% inhibition of the related *N.melanoleuca* phospholipase A_2 being observed at all dilutions of SPP tested. Significant inhibition of phospholipase A_2 activities derived from the more distantly related species were also observed at high concentrations of the SPP fraction, wherein 50% inhibition or *V.russelli* phospholipase A_2 was observed at a 1/25 dilution of SPP and a 50% inhibition of the *A.bilineatus* and *B.alternatus* phospholipase A_2 activities was observed at about a 1/12 dilution of SPP and a 50% inhibition of the *P.textilis* and *C.atrox* phospholipase A_2 activities was observed at about a 1/2-1/8 dilution of SPP.

These data indicate that the *N.scutatus* venom phospholipase A_2 inhibitor is a broad-spectrum inhibitor of snake venom phospholipase A_2 enzymes.

EXAMPLE 4

Inhibition of non-snake venom phospholipase A_2 enzymes by *N.scutatus* phospholipase A_2 inhibitor

Phospholipase A_2 enzyme activity assays were performed as described in Example 2. The assay was performed as above except that 10pPG (1-hexadecanoyl-2-(1-predecanoyl)-sn-glycero-3-phosphoglycerol, ammonium salt) was used as the substrate, because most of the non-snake venom PLA_2's are not active on 10pPC. Also, saline, rather than water was used for the negative control.

PLA_2 enzymes were diluted to achieve an enzyme activity sufficient to produce a change of 250 fluorescent units over 70-80 seconds in the enzyme assay, in the absence of inhibitor. Samples tested were; *N.scutatus* venom (positive control), bee venom phospholipase A_2 (*Apis meliffera*), porcine pancreatic phospholipase A_2 PLA_2 (*Sus scrofa*), and osteo-arthritis synovial fluid aspirates and rheumatoid arthritis-synovial fluid aspirates. Dilutions of phospholipase A_2-containing samples which were used were as follows; *N.scutatus* venom 1/30, bee venom phospholipase A_2 1/400, porcine pancreatic phospholipase A_2 1/3, all 1mg/ml. Osteo-arthritis, undiluted to 1/10 and rheumatoid-arthritis-synovial, 1/30, 25-

36mg/mL total protein. It should be noted that not all of the OA or RA samples meet with the activity criteria of 250 fluorescent intensity units over 70-80 seconds, however, the activity was consistent and measurable.

Dilutions of the SPP varied according to the phospholipase A_2 tested. Two dilution groups were used for a 7.13mg/mL solution of the SPP: Group 1; 1/14, 1/50, 1/330 and 1/660. Phospholipase A_2 sources challenged with this group were *N.scutatus* venom, porcine pancreatic phospholipase A_2 and bee venom phospholipase A_2. Group 2; ½, 1/7, 1/14 and 1/50. Phospholipase A_2 sources challenged with this group were, all OA and RA samples.

As shown in Figure 3a, the SPP fraction of *N.scutatus* phospholipase A_2 inhibitor strongly inhibited bee venom phospholipase A_2 at all concentrates tested. A 50% inhibition of porcine pancreas phospholipase A_2 was observed at a 1/4 dilution of SPP.

As shown in Figure 3b, the SPP fraction of *N.scutatus* phospholipase A_2 inhibitor significantly inhibited the three osteoarthritis samples tested, with about 40-60% inhibition of enzyme activity being observed at a 1/2 dilution of SPP. In two of the three samples tested, about 50% inhibition of phospholipase A_2 activity was observed at the 1/7 dilution level of SPP. Weak, albeit detectable inhibition of phospholipase A_2 in the rheumatoid arthritis sample tested was also detected at the 1/2-1/8 dilution of SPP.

These data indicate that the *N.scutatus* venom phospholipase A_2 inhibitor is a broad-spectrum inhibitor of non-snake venom-derived phospholipase A_2 activities.

EXAMPLE 5

Mixed micelle assay of recombinant human type II phospholipase A_2 and inhibition of enzyme activity using *N.scutatus* phospholipase A_2 inhibitor

An alternative assay of phospholipase A_2 activity was a mixed micelle phosphatidylethanolamine (PE/sodium deoxycholate (DOC) assay modified from a method of

Seilhamer *et al.* (1989). This assay is particularly suited to quantifying recombinant human phospholipase A_2 activity as it utilises a PE/DOC substrate. The PE substrate was prepared by dissolving freshly desiccated [^{14}C]PE (Amersham) in 2% w/v DOC, then diluting this to 0.22μmoles PE and 0.04% w/v DOC per sample in assay buffer (50mM Tris-HCl, pH 8.5, 2mM $CaCl_2$, 150mM NaCl, 0.04% w/v DOC). The sample was prepared by mixing 10μL of the test material with 10μL 10mM Tris-HCl, pH 7.4 and incubating for 10 minutes at 37°C. The reaction was started by the addition of 25μL pre-warmed substrate and terminated by the addition of 10μL 100mM EDTA. The reaction mixture (30μL) was spotted and dried onto silica TLC plates. The plates were chromatographed using chloroform:methanol:acetic acid (90:10:1) as solvent. The dried plates were then exposed overnight with Kodak X-OMAT AR film. Radioactivity at the origin was counted and the percent hydrolysis by phospholipase A_2 determined.

As shown in Figure 4, the recombinant human phospholipase A_2 activities is significantly inhibited at 0.1-1.0μM concentrations of *N.scutatus* phospholipase A_2 inhibitor. The IC_{50} of *N.scutatus* phospholipase A_2 inhibitor for recombinant human non-pancreatic phospholipase A_2 is approximately 1.5μM.

EXAMPLE 6

pH Optimum and temperature stability of *N.scutatus* venom phospholipase A_2 inhibitor

The pH stability was investigated by altering the pH of the solution in which the SPP (0.4mg/mL) was dissolved and then testing this in the phospholipase A_2 assay. The assay was performed as described in Example 2, using *N.scutatus* venom as the phospholipase A_2 source (1/200 dilution of a 1mg/mL with 10pPC as substrate). All samples were performed in triplicate with appropriate positive and negative controls. The pH values tested were: 2, 4, 6, 7, 8, 9, 10 and 12.

The temperature stability was assessed in the same manner as the pH stability. Samples were heated, or cooled, at the appropriate temperature and then immediately tested in the

phospholipase A_2 assay. Temperatures examined were; 4°C, 25°C, 37°C, 50°C, 60°C, 70°C, 80°C, 90°C and 100°C.

For both experiments samples were not preincubated with the venom as the stability of the phospholipase A_2 under the varying pH and temperature values could not be assured. However, the ratios phospholipase A_2 to inhibitor used in the preceding Examples were maintained in this procedure.

As shown in Figure 5a, NSI was stable in the pH range 4.0-12.0, with activity declining at extreme acidic pH values. Figure 5b shows the temperature stability of the inhibitor at all temperatures tested. Thus, NSI is a highly-stable protein.

EXAMPLE 7

Activity of the *N.scutatus* phospholipase A_2 inhibitor following de-glycosylation of the α-chain

The α-chain was deglycosylated with N-glycosidase F (cleaves N-linked sugars) or O-glycosidase (cleaves O-linked sugars) as follows: 10μg (10μL) of the SPP was denatured with an equal volume of 1% (w/v) SDS followed by boiling for 2 minutes. To this 90μL 20mM sodium phosphate buffer, pH 7.2, 50mM EDTA, nonidet P-40, 0.5% v/v was added followed by a further 2 minutes boiling. The SPP was then incubated with 0.4U N-glycosidase or 2.5mU O-glycosidase for 16 hours at 37°C. A sample was then run on SDS-PAGE under reducing conditions. The gel was then blotted onto nitrocellulose and sugar residues detected with the Boehringer Mannheim DIG glycan detection kit as per manufacturers instructions. Appropriate controls were performed. A duplicate gel was run and silver stained to determine the shift in molecular weight of the α-chain following deglycosylation.

It was determined that only N-linked sugars were present on the α-chain. As such, the α-chain was deglycosylated with N-glycosidase F as outlined above except that SDS and nonidet P-40 were omitted as were the boiling steps. This was to ensure that NSI was not

irreversibly denatured by boiling or SDS treatment. Deglycosylation was confirmed with the DIG glycan detection kit and the shift in molecular weight following SDS-PAGE. The sample was then assayed for inhibitory activity on *N.scutatus* venom (1/300 dilution of 1mg/mL solution dissolved in saline/0.1% w/v BSA) as described in Example 3. Native NSI was used as the positive control.

The formation of the NSI intact complex following deglycosylation of the α-chain was determined using size exclusion chromatography. The deglycosylated SPP (containing NSI) was run on a Superdex 75 column (3.2mm x 30mm) using the Pharmacia SMART HPLC system in 0.1M NH_4OAc pH 7.0. The column was calibrated with molecular weight standards. Native SPP was run as a positive control.

As shown in Figure 6a, de-glycosylated NSI retained activity compared to the native inhibitor, consistent with observations in respect of both *A.bilineatus* and bee venom phospholipase A_2 inhibitors.

However, the de-glycosylated NSI exhibited a different elution profile from Superdex 75 compared to the native inhibitor, with significantly higher molecular weight species being present, possible due to the formation of functional high molecular weight aggregates involving the de-glycosylated α-chain (Figure 6b). Additionally, the size of the assembled NSI complex differed slightly from native NSI (Figure 6b) due to the altered glycosylation status of the assembled complex.

EXAMPLE 8

Determination of the *N.scutatus* phospholipase A_2 inhibitor complex formation with notexin

The native molecular weight of NSI was determined using size exclusion chromatography using a Pharmacia Superose 12 HR10/30 column attached to a Waters 600 series HPLC system. Elution buffer was 0.1M NH_4OAc, pH 7.0 at a flow rate of 0.5mL/min. NSI (60μg) was loaded on the column. The column was calibrated with molecular weight standards. The

formulation of a stable complex between NSI and notexin was also investigated using size exclusion chromatography. The SPP (150μg) and notexin (100μg) were incubated for 30 minutes followed by elution on the Superose column. As shown in Figure 7, the NSI and notexin mixture eluted from Superose 12 immediately before NSI, confirming the ability of NSI to bind to notexin.

The peaks were collected and components identified by SDS-PAGE followed by silver staining to confirm their identities.

EXAMPLE 9

Amino acid sequence determination of the α-chain of *N.scutatus* phospholipase A_2 inhibitor

The α-chain was prepared essentially as outlined in the following Example (Example 10) for the β-chain, except that the α-chain was subjected to cleavage by either trypsin or endoproteinase Glu-C. The endoproteinase Glu-C and trypsin digestion profiles of the α-chain are presented in Figures 8 and 9, respectively. The amino acid sequences of various isoforms of the NSI α-chain are shown in SEQ ID NOs:1-3 and 9-11 (see Table 2).

EXAMPLE 10

Amino acid sequence determination of the β-chain of *N.scutatus* phospholipase A_2 inhibitor

The β-chain was purified from the SPP using RP-HPLC. A RP-300 7μm AQUAPORE (Brownlee Labs) column was connected to a Waters 600 series HPLC system. The buffers, 0.1% v/v trifluroacetic acid (TFA) and 80% v/v acetonitrile, 0.08% v/v TFA were filtered before use. The column was equilibrated in 0.1% v/v TFA at a flow rate of 0.5 mL/min. SPP (200μL; 200μg) was loaded onto the column, after centrifugation at 10,000 x *g* for 10 minutes (Eppendorf Centrifuge 5415C), and eluted with a gradient of 80% v/v acetonitrile, 0.08% v/v TFA over 65 minutes. The gradient developed as follows; 0% for first 3 minutes, 0-40% over next 7 minutes, no charge for 5 minutes, 40-80% over next 40 minutes 80-100%

eluting buffer over the remaining 10 minutes. All gradients were linear.

The purified β-chain was then reduced and carboxymethylated. Reduction was performed by lyophilising the protein to near dryness followed by resuspension in 100μL 10mM DTT, 50mM Tris-HCl, pH 8.0 and then heated for 10 min at 60°C then 20 min at 37°C. Alkylation was performed with the addition of 4-vinylpyridine to the above mixture to yield a final concentration of 1.5% v/v then incubate for 30 min at room temperature in the dark. The sample was desalted by narrow bore RP-HPLC.

After desalting, the sample was lyophilised and resuspend in 50mM NH_4HCO_3, pH 7.8 to which trypsin (Promega) was added at a 1:30 ratio (enzyme to protein). The protein was then incubated for 18 hours at 37°C. The digestion was stopped by the addition of TFA to a final concentration of 10% v/v. The peptides were then separated by RP-HPLC (C18, 2.1 x 100mm) on a Pharmacia SMART system. The resulting purified peptides were then N-terminally sequenced. The reduced and alkylated β-chain was also subjected to cleavage with cyanogen bromide (CNBr). The desalted protein was lyophilised and resuspended in 70% v/v formic acid. To this 0.5 mg CNBr was added and incubated for 20 hours at room temperature. The resulting peptides were separated as described above and then N-terminally sequenced. (Figure 7). The amino acid sequence of the β-chain is shown in SEQ ID NO:4 and the leader sequence is shown in SEQ ID NO:12 (Table 2).

EXAMPLE 11

Isolation and characterisation of a cDNA clone encoding *N.scutatus* phospholipase A_2 inhibitor

Production of cDNA library

A cDNA library was produced from *N.scutatus* liver. The liver was collected and immediately placed in liquid nitrogen. A piece was taken and total RNA isolated using a PolyA tract mRNA isolation system (Promega) as per manufacturers instructions. From this cDNA was produced using a cDNA synthesis System Plus kit (Amersham) as per

instructions. The cDNA was then adapted with *EcoRI* adaptors and size selected using the Riboclone *EcoRI* adaptor ligation system I (Promega). The size selected cDNA was then used for the production of a cDNA library in the bacteriophage λZAPII (*EcoRI* digested and CIAP treated). The library was then titred and amplified as per instructions.

Screening of library, PCR and oligomers

Degenerate primers were designed from known N-terminal sequence of the α-chain and internal peptide data. Homology with the PLI from *Crotalus durissus terrificus* was used to aid in the primer design. The DNA sequence was known for the Crotalid PLI and as such minimally degenerate primers could be designed. cDNA prepared from *N.scutatus* liver was used as the template for the PCR. The forward primer sequence is 5′-CCAGAAGATCAG/ATGTGGC-3′ [SEQ ID NO:91] and the reverse primer sequence is 5′-ATIGCGATGTCTCCAGG-3′ [SEQ ID NO:92]. PCR was performed in a Hybaid Omni-Gene PCR machine with the following cycle conditions [95°C x 2min] x 1, [61°C x 60sec, 72°C x 45sec, 95°C x 30sec] x 34, using tube temperature control and *Taq* polymerase (Qiagen). Product size and purity was assessed with TBE-PAGE. Ubiquitin primers were used as positive controls.

Using these primers, a specific 357bp product was amplified. The product was sequenced [SEQ ID NO: 5] using automated Li-cor sequencers. The sequence was confirmed to be the α-chain. As such, this product was used as a probe for the cDNA library. The product was labelled with a DIG labelling kit (Boehringer Mannheim) to enable detection of the probe. The library was then screened as per instructions. Three rounds of screening were performed.

To establish the DNA sequence for the α-chain, 5′ and 3′ RACE was performed. The Marathon cDNA Amplification Kit (Clonetech) was used as per manufacturers protocol. Degenerate primers, as used in Example 11, were used with the manufacturers supplied PCR primer for either 5′ or 3′ RACE. For 5′ RACE the PCR protocol was as follows: [95°C x 2.5 mm] x 1, [94°C x 30s, 61°C x 45s, 72°C x 2 min] x 34. For 3′ RACE the annealing

temperature (61°C) was increased to 65°C, all other parameters remained the same. After amplification of the partial gene it was sequenced using automated Li-cor DNA sequencers. The various isoforms of the α chain are shown in SEQ ID NOs: 5-7 and of the β chain in SEQ ID NO:8 (Table 2). Table 2 also summarizes the SEQ ID NOs for the leader sequences from the α-chain and β-chain of NSI and its isoforms.

EXAMPLE 12

In vivo protection against notexin by NSI

The SPP was again tested against notexin to evaluate the effectiveness with which NSI was able to protect *in vivo*. Neonatal QS mice (3-5g) were used as the assay system, neonates are used in preference to adult mice as they are a more sensitive assay and also to conserve the limited amount of inhibitor available.

First, a toxic dose (TD) was established, by testing dilutions above and below the published LD_{50} values for notexin [0.188mg/kg in saline, injected sub-cutaneously (S.C.); Sutherland, (1990)]. The dose was given in a total volume of 50μL injected s.c., the mice were incubated for 17 hours on a heating pad maintained at 30°C. The TD determined for notexin was 0.015μg/mouse. The TD was then used in the protection studies.

Protection studies were performed in triplicate with the following samples; 1x, 2x, 3x and 4x TD plus SPP (1mg/mL), positive control (TD plus saline), SPP control (SPP plus saline) and two negative controls (human serum and BSA, each at a concentration of 1mg/mL, plus TD). 160 μL toxin was added to 160μL of the appropriate sample, or 160μL SPP plus 160μL saline, and incubated at 37°C for 1 hour. A total volume of 100μL was then injected into each mouse. Mice were incubated as described above.

As shown in Table 5, the SPP fraction of *N.scutatus* phospholipase A_2 inhibitor successfully protected mice against up to at least 4xTD of notexin.

TABLE 5

In vivo protection of mice against notexin using *N.scutatus* venom phospholipase A_2 inhibitor

Sample Tested	Protection (alive/total)
4TD + SPP	3/3
3TD + SPP	3/3
2TD + SPP	3/3
TD + SPP	3/3
BSA + TD	0/3
Human Serum + TD	0/3
Venom Control (TD)	0/3
SPP Control	3/3

Samples were as follows: 1x, 2x, 3x and 4xTD plus SPP (1mg/mL); BSA, BSA (1mg/mL) plus TD; human serum, human serum (1mg/mL) plus TD; venom control, TD plus saline; SPP control, SPP plus saline. Equal volumes of each were incubated at 37°C for 1 hour before injection. TD=0.015μg notexin s.c.

EXAMPLE 13

Crystallisation experiments

Protein solutions of greater than 95% purity and between 5-50 mg/ml are surveyed for crystallisation parameters by the handing drop vapour diffusion method. The method involves centrifugation of the protein solution to remove particulates; the setting up of reservoirs containing different buffers, precipitants and additives in multiwell plates; pipetting 2μl of reservoir solution onto cover slips plus 2μl of protein solution to the drop; inverting the coverslip over the well; and incubating and observing for crystal formation.

Examples of precipitants include salts such as ammonium sulphate, ammonium formate and sodium citrate; different molecular weight polyethylene glycol [PEG 4000, 3000, 8000,

20000]; organic solvents [MPD, ethanol] and mixtures of these.

Additives tested may include 0.25-1% v/v non-ionic detergent [eg. β-octyl glucoside]; dioxane; metal ions such as Ca^{2+}, Zn^{2+}; reducing agents [dithiothreitol] and glycerol.

Other variables include pH, buffer type and temperature, amongst others.

As well as testing for crystal formation of purified NSI, co-crystallisation of NSI and PLA_2 is investigated to determine the structure of the interactive site. The two proteins are mixed in equimolar quantities and the parameter survey procedure described above repeated.

Parameters which produce crystals are used with greater quantities of protein to produce large crystals suitable for X-ray diffraction experiments. These crystals are mounted, exposed to an X-ray source and diffraction data collected.

The data obtained are manipulated by phase determination, phase improvement extension, followed by interpretation of the electron density map. This process is aided by the amino acid sequence data and at 3A0 resolution, an atomic model can be constructed. The atomic model of the co-crystal structure is based in part on the known solution of the human type II PLA2 structure. The atomic model can be refined by various procedures including energy minimisation.

EXAMPLE 14

Use of Crystal Structure Data

The crystal structures, particularly of the co-crystallised inhibitor and PLA_2 permits the determination of the structure of the interactive site. At the gross level, the molecular surfaces which make contact or are in close proximity are visualised, while at the finest resolution of the structure, hydrophobic and ionic interactions between specific amino acid side chains are determined. This information specifies the critical residues of both the NSI

and PLA_2 molecules which involved the protein-protein interaction resulting in PLA_2 inhibitory activity.

This atomic model is used to model further the possible interaction between human type II PLA_2 and the other snake PLA_2 inhibitors for which amino acid sequence data is obtained by the present inventors. These models of the interaction between the PLA_2 and the inhibitors are compared to their biological activity to gain a greater understanding of the inhibitory mechanism. Detailed information on the molecular interactions of these proteins, in particular elucidation of the structure of the interactive site, assists the rational design of synthetic proteins, peptides and organic molecules which utilise the inhibitory mechanism of NSI and other phospholipase inhibitory proteins of the invention.

EXAMPLE 15

Association (or Re-association) Experiments

Using native NSI, the α and β chains are separated and purified, then mixed under various conditions (e.g. guandinium chloride) to promote the re-association of the chains into the native tetrameric structure. The inhibitory activity of the re-associated molecule is tested. This information is used to produce novel active recombinant phospholipase inhibitors; based upon the α-chain and β-chain sequences of NSI.

EXAMPLE 16

Mixing Experiments

The conditions which permit reassociation of separated α and β chains are used in mixing experiments, using purified α- and β- chains from homologues of native phospholipase inhibitor polypeptides of the invention. For example, the α-chain from the tiger snake combined with the β-chain from either the Inland Taipan or Brown Snake. All permutations are produced and tested for inhibitory activity. Novel inhibitory proteins, having altered specificity or activity are thus obtained.

Similar combinatorial mixing is performed with expressed recombinant α- and β- chains and with combinations of native and recombinant α- and β- chains, to identify phospholipase inhibitors having altered specificity and/or activity.

EXAMPLE 17

Cloning of PLA_2 inhibition from *Notechis ater* and from *Psuedonaja textilis*

Using similar procedures as described above, the PLA_2 inhibitor gene from *N. ater* (NAI) and *P. textilis* (PII) where cloned.

The nucleotide sequence of NAI α chain, isoforms i (NAIαi), ii (NATαii) and v (NAIαv) are shown in SEQ ID NOs:39-41, respectively. The nucleotide sequence of the leader sequence is shown in SEQ ID NO:47 (NAIαiL), 48 (NAIαiiL) and 49 (NAIαivL). The nucleotide sequence of the β-chain is shown in SEQ ID NO:42 (NAIβ) and its leader sequence is shown in SEQ ID NO:50 (NAIβl).

The corresponding amino acid sequences are shown in SEQ ID NOs: 17-38 and 43-46 (see Table 2).

The nucleotide and amino acid sequences of the PLA_2 inhibitor from *P. textilis* (PTI) are shown in SEQ ID NOs as summarized in Table 2.

EXAMPLE 18

Isolation of the gene encoding the alpha chain of PLA_2 inhibitor from coastal and inland taipan snakes

Total RNA was prepared from the liver of both Coastal Taipan and Inland Taipan snakes. Isolation of mRNA was carried out using standard techniques and purified mRNA was then transcribed into cDNA using the Reverse Transcriptase enzyme.

Primers for the isolation of the α-chain of the inhibitor protein were designed by using the

DNA sequence previously determined for the α-chain of NSI (Tiger snake). A forward primer (a) was designed to include the start codon at the beginning of the secretion signal and a restriction site to allow directional cloning of the gene into the expression vector. The reverse primer (b) was complementary to the carboxy terminus of the protein and contained the translational stop codon:

(a) Forward primer (Tai for): 5′- CGCGGATCCATGAAATCCCTA-3′ [SEQ ID NO:93]

(b) Reverse primer (Tai rev): 5′- CCGGAATTCTTATTATTCAGAAGG-3′ [SEQ ID NO:94]

Amplification of the gene encoding the α-chain was carried out under standard conditions using the forward and reverse primers and the mRNA/cDNA hybrid as template. An amplified DNA product of approximately 610 bp was produced and isolated by agarose gel purification. The purified DNA was ligated into the sequencing vector pGEM-T and sequenced on an automated DNA sequencer.

The amino acid and nucleotide sequences of the coastal taipan (*O. scutellatus*) phospholipase inhibitor (OSI) are presented in SEQ ID NOs:51-53, 57-59, 54-56, 60-62 and 54-62. The amino acid and nucleotide sequences of the inland taipan (*O. microlepidotus*) phospholipase inhibitor (OMI) are presented in SEQ ID NOs:63-66, 71-74, 67-70 and 75-78. In both cases, the secretion signal consists of about 19 amino acids and this sequence is removed during processing of the protein.

The amino acid sequence alignment of the isoforms of NSI, NAI, OSI, OMI and PTI (α- and β- chains) is shown in Figure 11.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT (or than US): HSC (PLA) Pty Ltd AND Flair (PLA) R&D Pty Ltd AND Active (PLA) R&D Pty Ltd AND Heracles (PLA) R&D Pty Ltd AND Apelda (PLA) R&D Pty Ltd AND Edzell (PLA) R&D Pty Ltd AND Northmoor (PLA) R&D Pty Ltd

(US only): BROADY Kevin William, HAINS Peter Gregory

(ii) TITLE OF INVENTION: PHOSPHOLIPASE INHIBITOR

(iii) NUMBER OF SEQUENCES: 94

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(F) ZIP: 3000

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: INTERNATIONAL/PCT
(B) FILING DATE: 27-NOV-1998

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PP0767
(B) FILING DATE: 5-DEC-1997

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 182 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

His	Ser	Cys	Glu	Ile	Cys	His	Asn	Phe	Gly	Lys	Asp	Cys	Gln	Ser	Asp
1				5					10					15	
Glu	Thr	Glu	Glu	Cys	Ala	Ser	Ala	Glu	Asp	Gln	Cys	Gly	Thr	Val	Leu
			20					25					30		
Met	Glu	Val	Ser	Ser	Ala	Pro	Ile	Ser	Phe	Arg	Ser	Ile	His	Arg	Lys
		35					40					45			
Cys	Phe	Ser	Ser	Ser	Ile	Cys	Lys	Leu	Glu	Arg	Phe	Asp	Ile	Asn	Ile
	50					55					60				
Gly	His	Asp	Ser	Tyr	Leu	Arg	Gly	Arg	Ile	His	Cys	Cys	Asp	Glu	Ala
65					70					75					80
Arg	Cys	Glu	Ala	Gln	Gln	Phe	Pro	Gly	Leu	Pro	Leu	Ser	Phe	Pro	Asn
				85					90					95	
Gly	Tyr	His	Cys	Pro	Gly	Ile	Leu	Gly	Val	Phe	Ser	Val	Asp	Ser	Ser
			100					105					110		
Glu	His	Glu	Ala	Ile	Cys	Arg	Gly	Thr	Glu	Thr	Lys	Cys	Ile	Asn	Leu
		115					120					125			
Ala	Gly	Phe	Arg	Lys	Glu	Arg	Tyr	Pro	Ile	Asp	Ile	Ala	Tyr	Asn	Ile
	130					135					140				
Lys	Gly	Cys	Thr	Ser	Ser	Cys	Pro	Glu	Leu	Arg	Leu	Asn	Arg	Thr	His
145					150					155					160

Glu Glu His Gly Asn Gly Leu Ile Lys Val Glu Cys Thr Glu Ala Ser
165 170 175

Lys Ile Thr Pro Ser Glu
180

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 182 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

His Ser Cys Glu Ile Cys His Asn Phe Gly Lys Asp Cys Gln Ser Glu
1 5 10 15

Glu Ala Lys Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly Thr Val Leu
20 25 30

Met Glu Val Ser Ser Ala Pro Ile Ser Phe Arg Thr Ile His Arg Asn
35 40 45

Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu Arg Phe Asp Ile Asn Ile
50 55 60

Gly His Asp Ser Tyr Leu Arg Gly Arg Ile His Cys Cys Asp Glu Ala
65 70 75 80

Arg Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
85 90 95

Gly Tyr His Cys Pro Gly Ile Phe Gly Val Phe Ser Val Asp Ser Ser
100 105 110

Glu His Glu Ala Ile Cys Arg Gly Ser Glu Thr Lys Cys Ile Lys Ile
115 120 125

Ala Gly Phe Arg Asn Glu Arg Phe Phe Gly Asp Met Gly Tyr Asn Ile

130 135 140

Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Lys Leu Asn Arg Thr His
145 150 155 160

Glu Glu His Gly Asn Gly Leu Ile Lys Val Glu Cys Thr Glu Ala Ser
165 170 175

Lys Ile Thr Pro Ser Glu
180

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 183 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

His Ser Cys Glu Ile Cys His Asn Leu Gly Arg Asp Cys Glu Thr Glu
1 5 10 15

Glu Ala Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly Thr Val Leu
20 25 30

Met Glu Val Ser Ser Ala Pro Ile Ser Phe Arg Ser Ile His Arg Asn
35 40 45

Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu Arg Phe Asp Ile Asn Ile
50 55 60

Gly His Asp Ser Tyr Leu Arg Gly Arg Ile His Cys Cys Asp Glu Ala
65 70 75 80

Arg Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
85 90 95

Gly Tyr His Cys Pro Gly Ile Leu Gly Val Phe Ser Val Asp Ser Ser
100 105 110

Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys Ile Asn Leu
115 120 125

Ala Gly Phe Arg Lys Glu Arg Phe Pro Gly Asp Ile Gly Tyr Asn Ile
130 135 140

Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Ser Asn Arg Thr
145 150 155 160

His Glu Glu Asp Arg Asn Asp Leu Ile Lys Val Glu Cys Thr Asp Ala
165 170 175

Ser Lys Ile Thr Pro Ser Glu
180

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 181 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Leu Glu Cys Glu Ile Cys Ile Gly Leu Gly Leu Glu Cys Asn Thr Trp
1 5 10 15

Thr Lys Thr Cys Asp Ala Asn Gln Asp Thr Cys Val Thr Phe Gln Thr
20 25 30

Glu Val Ile Arg Ala Pro Val Ser Leu Ser Leu Ile Ser Lys Ser Cys
35 40 45

Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Val Glu Thr Ser Pro His
50 55 60

Asn Glu Leu Thr Val Lys Thr Lys Arg Thr Cys Cys Thr Gly Glu Glu
65 70 75 80

Cys Lys Thr Leu Pro Pro Pro Val Leu Gly His Lys Val Asn Pro Pro

85 90 95

Asn Gly Leu Gln Cys Pro Gly Cys Leu Gly Leu Ser Ser Lys Glu Cys
100 105 110

Thr Glu His Leu Val Ser Cys Arg Gly Ser Glu Asn Gln Cys Leu Ser
115 120 125

Ile Ile Gly Lys Glu Phe Gly Leu Phe Phe Arg Ala Leu Ser Tyr Lys
130 135 140

Gly Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Arg Phe Trp
145 150 155 160

Asn Val Leu Glu Asp Val Glu Val Asp Phe Lys Cys Thr Pro Ala Leu
165 170 175

Pro Lys Ser Ser Gln
180

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 500 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CACTCATGTG AAATTTGTCA CAATTTTGGA AAAGATTGCC AGAGTGACGA GACAGAGGAA 60

TGTGCCTCTG CAGAAGATCA ATGTGGCACG GTGTTGATGG AGGTTTCATC AGCACCTATT 120

TCCTTCCGAT CCATTCATAG GAAGTGTTTC TCATCCAGCA TCTGCAAACT TGAACGCTTT 180

GATATAAATA TTGGACATGA TTCCTATTTG AGAGGAAGAA TCCACTGTTG TGATGAAGCA 240

AGGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC 300

CCTGGCATTC TTGGTGTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA 360

ACTGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAAAG AAAGATATCC TATAGACATT 420

GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAA TAGAACTCAC 480

GAAGAACATG GAAATGGTCT 500

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 500 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CACTCATGTG AAATTTGTCA CAATTTTGGA AAAGACTGCC AGAGTGAGGA GGCAAAGGAA 60

TGTGCGTCTC CAGAAGATCA ATGTGGCACG GTGTTGATGG AGGTTTCATC AGCACCTATT 120

TCCTTCCGAA CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAACT TGAACGCTTT 180

GATATAAATA TTGGACATGA TTCCTATTTG AGAGGAAGAA TCCACTGTTG TGATGAAGCA 240

AGGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC 300

CCTGGCATTT TTGGTGTATT CTCAGTGGAC AGTTCTGAAC ATGAAGCTAT TTGCAGAGGA 360

AGTGAAACCA AATGCATTAA AATTGCGGGA TTCAGAAACG AAAGATTTTT TGGAGACATG 420

GGTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAAGTTGAA TAGAACTCAC 480

GAAGAACATG GAAATGGTCT 500

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 500 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CACTCATGTG AAATTTGTCA CAATTTGGGA AGAGATTGTG AGACTGAGGA GGCAGAGGAA 60

TGTGCCTCTC CAGAAGATCA ATGTGGCACG GTGTTGATGG AGGTTTCATC AGCACCTATT 120

TCCTTCCGAT CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAACT CGAACGCTTT 180

GATATAAATA TTGGACATGA TTCCTATTTG AGAGGAAGAA TCCACTGTTG TGATGAAGCA 240

AGGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC 300

CCTGGCATTC TTGGTGTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA 360

ACTGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAAAG AAAGATTTCC TGGAGACATC 420

GGTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAG CAATAGAACT 480

CACGAAGAAG ATAGAAATGA 500

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 500 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CTTGAGTGTG AGATTTGTAT CGGGCTGGGC CTGGAATGTA ACACCTGGAC GAAAACCTGT 60

GATGCTAATC AAGATACTTG TGTTACCTTT CAAACTGAAG TGATAAGAGC CCCTGTGTCC 120

CTCTCTTTGA TCTCAAAATC CTGTGGTACT TCTGACACTT GCCATCTTAA CTACGTGGAG 180

ACGAGTCCAC ATAATGAACT AACAGTGAAG ACCAAAAGAA CCTGCTGTAC TGGGGAGGAA 240

TGTAAAACTC TGCCACCGCC TGTGCTTGGA CACAAAGTCA ACCCACCCAA CGGACTTCAG 300

TGTCCTGGAT GCCTTGGATT GTCCTCAAAA GAATGCACTG AACACCTGGT TTCCTGCCGG 360

GGATCTGAAA ACCAGTGTTT GTCTATAATT GGGAAGGAAT TTGGCCTTTT CTTCAGAGCA 420

TTGTCTTATA AAGGATGTGC TACGGAGAGT CTGTGCACTT TATTTGAGAA GAGGTTCTGG 480

AATGTTTTAG AGGATGTTGA 500

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg

1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Lys Ser Leu Leu Phe Cys Cys Leu Phe Gly Thr Phe Leu Ala Thr
1 5 10 15

Gly Met Cys

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ATGAAGTCCC TCTTATTCTG TTGCCTCTTT GGCACTTTCT TAGCTACAGG CATGTGT 57

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

His Ser Cys Glu Ile Cys His Asn Phe Gly Arg Asp Cys Gln Ser Asp
1 5 10 15

Glu Ala Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly
20 25

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

His Ser Cys Glu Ile Cys His Asn Leu Gly Lys Asp Cys Glu Thr Glu
1 5 10 15

Glu Thr Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly
20 25

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ile Thr Pro Ser Glu
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Arg Phe Asp Ile Asn Ile
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Ile Asn Leu Ala Gly Phe
1 5

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Ala Ser Lys Ile Thr Pro Ser Glu
1 5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Tyr Pro Gly Asp Ile Ala Ile
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Leu Glu Cys Glu Ile Cys Ile Gly Leu Gly Leu Glu Cys Asn Thr Trp
1 5 10 15

Thr Lys Thr Cys Asp Ala Asn Gln Asp Thr Cys Val
20 25

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Ala Leu Ser Tyr Lys
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Ser	Cys	Gly	Thr	Ser	Asp	Thr	Cys	His	Leu	Asn	Tyr	Val	Glu	Thr	Thr
1				5					10					15	

Pro His Asn

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Thr	Cys	Asp	Ala	Asn	Gln	Asp	Thr	Cys	Val	Thr	Phe	Gln	Thr	Glu	Val
1				5					10					15	

Ile Arg

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ala Pro Val Thr Leu Gly Leu Ile
1 5

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Glu Cys Thr Glu His Leu Val Ser Cys Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Phe Trp Asn Val Leu Glu Asp Val Glu Val Asp Phe Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Gly Ser Glu Asn Gln Cys Lys Ser Ile Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Val Asn Pro Pro Asn Gly Leu Gln Cys Pro Gly Cys Leu Gly Leu Ser
1 5 10 15

Ser Leu Glu Cys Thr Glu
20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Cys Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Val Glu Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Glu Phe Gly Leu Phe Phe Arg
1 5

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 183 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

His Ser Cys Glu Ile Cys His Asn Phe Gly Lys Asp Cys Glu Gly Gly
1 5 10 15

Glu Thr Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly Thr Val Leu
20 25 30

Met Glu Val Ser Thr Ala Pro Ile Ser Phe Arg Ser Ile His Arg Asn
35 40 45

Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu Arg Phe Asp Ile Asn Ile
50 55 60

Gly His Asp Ser Phe Leu Arg Gly Arg Ile His Cys Cys Asp Glu Ala
65 70 75 80

Arg Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
85 90 95

Gly Tyr His Cys Pro Gly Ile Leu Gly Leu Phe Ser Val Asp Ser Ser
100 105 110

Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys Ile Asn Leu
115 120 125

Ala Gly Phe Arg Arg Glu Arg Phe Pro Gly Asp Ile Ala Tyr Asn Ile
130 135 140

Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Ser Asn Arg Thr
145 150 155 160

His Glu Glu His Arg Asn Asp Leu Ile Lys Val Glu Cys Thr Glu Ala
165 170 175

Ser Lys Asn Thr Pro Ser Glu
180

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 182 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

His Ser Cys Glu Ile Cys His Asn Phe Gly Lys Asp Cys Gln Ser Asp

1 5 10 15

Glu Thr Glu Glu Cys Ala Ser Ala Glu Asp Gln Cys Gly Thr Val Leu
20 25 30

Met Glu Val Ser Ser Ala Pro Ile Ser Phe Arg Ser Ile His Arg Lys
35 40 45

Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu Arg Phe Asp Ile Asn Ile
50 55 60

Gly His Asp Ser Tyr Leu Arg Gly Arg Ile His Cys Cys Asp Glu Ala
65 70 75 80

Arg Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
85 90 95

Gly Tyr His Cys Pro Gly Ile Leu Gly Val Phe Ser Val Asp Ser Ser
100 105 110

Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys Ile Asn Leu
115 120 125

Ala Gly Phe Arg Lys Glu Arg Tyr Pro Ile Asp Ile Ala Tyr Asn Ile
130 135 140

Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Asn Arg Thr His
145 150 155 160

Glu Glu His Arg Asn Asp Leu Ile Lys Val Glu Cys Thr Glu Ala Ser
165 170 175

Lys Ile Thr Pro Ser Glu
180

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 183 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
His Ser Cys Glu Ile Cys His Asn Phe Gly Lys Asp Cys Glu Gly Gly
1               5                  10                  15

Val Thr Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly Thr Val Leu
            20                  25                  30

Leu Glu Val Ser Thr Ala Pro Ile Ser Thr Arg Thr Ile His Arg Asn
        35                  40                  45

Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu Arg Phe Asp Ile Asn Ile
    50                  55                  60

Gly His Asp Ser Tyr Met Arg Gly Arg Ile His Cys Cys Asp Glu Ala
65                  70                  75                  80

Arg Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
                85                  90                  95

Gly Tyr His Cys Pro Gly Ile Leu Gly Leu Phe Ser Val Asp Ser Ser
            100                 105                 110

Glu His Glu Ala Ile Cys Arg Gly Ser Glu Thr Lys Cys Ile Lys Ile
        115                 120                 125

Ala Gly Phe Arg Arg Glu Arg Tyr Pro Ile Asp Ile Ala Tyr Asn Ile
    130                 135                 140

Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Ser Asn Arg Thr
145                 150                 155                 160

His Glu Glu His Arg Asn Asp Leu Ile Lys Val Glu Cys Thr Asp Ala
                165                 170                 175

Ser Lys Ile Thr Pro Ser Glu
            180
```

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 181 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Leu Glu Cys Glu Ile Cys Ile Gly Leu Gly Leu Glu Cys Asn Thr Trp
1 5 10 15

Thr Lys Thr Cys Asp Ala Asn Gln Asp Thr Cys Val Thr Phe Gln Thr
20 25 30

Glu Val Ile Arg Ala Pro Val Ser Leu Ser Leu Ile Ser Lys Ser Cys
35 40 45

Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Val Glu Thr Ser Pro His
50 55 60

Asn Glu Leu Thr Val Lys Thr Lys Arg Thr Cys Cys Thr Gly Glu Glu
65 70 75 80

Cys Lys Thr Leu Pro Pro Pro Val Leu Gly His Lys Val Asn Pro Pro
85 90 95

Asn Gly Leu Gln Cys Pro Gly Cys Leu Gly Leu Ser Ser Lys Glu Cys
100 105 110

Thr Glu His Leu Val Ser Cys Arg Gly Ser Glu Asn Gln Cys Leu Ser
115 120 125

Ile Ile Gly Lys Glu Phe Gly Leu Phe Phe Arg Ala Leu Ser Tyr Lys
130 135 140

Gly Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Arg Phe Trp
145 150 155 160

Asn Val Leu Glu Asp Val Glu Val Asp Phe Lys Cys Thr Pro Ala Leu
165 170 175

Pro Lys Ser Ser Gln
180

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 501 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CACTCATGTG AAATTTGTCA CAATTTTGGA AAAGATTGCG AGGGTGGGGA GACAGAGGAA 60

TGTGCCTCTC CAGAAGATCA ATGTGGCACA GTGTTGATGG AGGTTTCAAC AGCACCTATT 120

TCCTTCCGAT CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAACT TGAACGCTTT 180

GATATAAATA TTGGACATGA TTCCTTTTTG AGAGGAAGAA TCCACTGTTG TGATGAAGCA 240

AGGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC 300

CCTGGAATTC TTGGTTTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA 360

ACTGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAGAG AAAGATTTCC TGGAGACATC 420

GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAG CAATAGAACT 480

CACGAAGAAC ATAGAAATGA C 501

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 501 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CACTCATGTG AAATTTGTCA CAATTTTGGA AAAGATTGCC AGAGTGACGA GACAGAGGAA 60

TGTGCCTCTG CAGAAGATCA ATGTGGCACG GTGTTGATGG AGGTTTCATC AGCACCTATT 120

TCCTTCCGAT CCATTCATAG GAAGTGTTTC TCATCCAGCC TCTGCAAACT TGAACGCTTT 180

GATATAAATA TTGGACATGA TTCCTATTTG AGAGGAAGAA TCCACTGTTG TGATGAAGCA 240

AGGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC 300

CCTGGCATTC TTGGTGTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA 360

ACTGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAAAG AAAGATATCC TATAGACATC 420

GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAA TAGAACTCAC 480

GAAGAACATA GAAATGATCT A 501

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 501 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CACTCATGTG AAATTTGTCA CAATTTTGGA AAAGATTGCG AGGGTGGGGT GACAGAGGAA 60

TGTGCCTCTC CAGAAGATCA ATGTGGCACA GTGTTGCTGG AGGTTTCAAC AGCACCTATT 120

TCCACCCGAA CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAACT TGAACGCTTT 180

GATATAAATA TTGGACATGA TTCCTATATG AGAGGAAGAA TCCACTGTTG TGATGAAGCA 240

AGGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC 300

CCTGGCATTC TTGGTTTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA 360

AGTGAAACCA AATGCATTAA AATTGCGGGA TTCAGAAGAG AAAGATATCC TATAGACATC 420

GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAG CAATAGAACT 480

CACGAAGAAC ATAGAAATGA T 501

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 501 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CTTGAGTGTG AGATTTGTAT CGGGCTGGGC CTGGAATGTA ACACCTGGAC GAAAACCTGT 60

GATGCTAATC AAGATACTTG TGTTACCTTT CAAACTGAAG TGATAAGAGC CCCTGTGTCC 120

CTCTCTTTGA TTTCAAAATC CTGTGGTACT TCTGACACTT GCCATCTTAA CTACGTGGAG 180

ACGAGTCCAC ATAATGAACT AACAGTGAAG ACCAAAAGAA CCTGCTGTAC TGGGGAGGAA 240

TGTAAAACTC TGCCACCGCC TGTGCTTGGA CACAAAGTCA ACCCACCCAA CGGACTTCAG 300

TGTCCTGGAT GCCTTGGATT GTCCTCAAAA GAATGCACTG AACACCTGGT TTCCTGCCGG 360

GGATCTGAAA ACCAGTGTTT GTCTATAATT GGGAAAGAAT TTGGCCTTTT CTTCAGAGCA 420

TTGTCTTATA AAGGATGTGC TACGGAGAGT CTGTGCACTT TATTTGAGAA GAGGTTCTGG 480

AATGTTTTAG AGGATGTTGA A 501

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg

1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Met Lys Ser Leu Leu Phe Cys Cys Leu Phe Gly Thr Phe Leu Ala Thr
1 5 10 15

Gly Met Cys

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

ATGAAGTCCC TCTTATTCTG TTGCCTCTTT GGCACTTTCT TAGCTACAGG CATGTGT 57

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 183 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

His	Ser	Cys	Glu	Ile	Cys	Arg	Asn	Phe	Gly	Lys	Asp	Cys	Glu	Ser	Glu
1				5					10					15	

Glu	Ala	Glu	Glu	Cys	Ala	Ser	Pro	Glu	Asp	Gln	Cys	Gly	Thr	Val	Leu
			20					25					30		

Leu	Glu	Ile	Ser	Ser	Ala	Pro	Ile	Ser	Phe	Arg	Ser	Ile	His	Arg	Asn
		35					40					45			

Cys	Phe	Ser	Ser	Ser	Leu	Cys	Lys	Leu	Glu	His	Phe	Asp	Ile	Asn	Ile
	50					55					60				

Gly	His	Asp	Ser	Tyr	Val	Arg	Gly	Arg	Ile	His	Cys	Cys	Asp	Glu	Glu
65					70					75					80

Arg	Cys	Glu	Ala	Gln	Gln	Phe	Pro	Gly	Leu	Pro	Leu	Ser	Phe	Pro	Asn
				85					90					95	

Gly	Tyr	His	Cys	Pro	Gly	Ile	Leu	Gly	Ala	Phe	Ser	Val	Asp	Ser	Ser
			100					105					110		

Glu	His	Glu	Ala	Ile	Cys	Arg	Gly	Thr	Glu	Thr	Lys	Cys	Ile	Asn	Leu
		115					120					125			

Ala	Gly	Phe	Arg	Lys	Glu	Arg	Tyr	Pro	Val	Asp	Ile	Ala	Tyr	Asn	Ile
	130					135					140				

Lys	Gly	Cys	Thr	Ser	Ser	Cys	Pro	Glu	Leu	Lys	Leu	Ser	Asn	Arg	Thr
145					150					155					160

His	Glu	Glu	Arg	Arg	Asn	Asp	Leu	Ile	Thr	Leu	Glu	Cys	Thr	Asp	Ala

165 170 175

Ser Lys Ile Ala Pro Ser Glu
180

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 183 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Arg Ser Cys Glu Ile Cys His Asn Phe Gly Lys Val Cys Glu Ser Glu
1 5 10 15

Glu Ala Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly Thr Val Leu
20 25 30

Leu Glu Ile Ser Ser Ala Pro Ile Ser Phe Arg Thr Ile His Arg Asn
35 40 45

Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu His Phe Asp Ile Asn Ile
50 55 60

Gly His Asp Ser Tyr Ile Arg Gly Arg Ile His Cys Cys Asp Glu Glu
65 70 75 80

Lys Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
85 90 95

Gly Tyr His Cys Pro Gly Ile Leu Gly Val Phe Ser Val Asp Ser Ser
100 105 110

Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys Ile Asn Leu
115 120 125

Ala Gly Phe Arg Lys Glu Arg Tyr Pro Leu Asp Ile Ala Tyr Asn Ile
130 135 140

Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Ser Asn Arg Thr
145 150 155 160

His Glu Glu His Arg Asn Glu Leu Ile Lys Val Glu Cys Thr Asp Ala
165 170 175

Ser Lys Ile Thr Pro Ser Glu
180

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 181 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Leu Glu Cys Glu Ile Cys Ile Gly Leu Gly Arg Glu Cys Asn Ser Trp
1 5 10 15

Thr Lys Thr Cys Asp Ala Asn Gln Asp Thr Cys Val Thr Phe Gln Thr
20 25 30

Glu Val Ile Arg Ala Pro Val Ser Leu Ser Leu Ile Ser Lys Ser Cys
35 40 45

Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Val Glu Thr Ser Pro His
50 55 60

Asn Glu Leu Thr Val Lys Thr Lys Arg Thr Cys Cys Thr Gly Glu Glu
65 70 75 80

Cys Lys Thr Leu Pro Pro Pro Val Leu Gly Tyr Lys Val Asn Pro Pro
85 90 95

Asn Gly Leu Gln Cys Pro Gly Cys Leu Gly Leu Ser Ser Lys Glu Cys
100 105 110

Thr Glu His Pro Val Ser Cys Arg Gly Ser Glu Asn Gln Cys Leu Ser

115 120 125

Ile Ile Gly Lys Glu Phe Gly Leu Phe Phe Arg Ala Leu Ser Tyr Lys
130 135 140

Gly Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Arg Phe Trp
145 150 155 160

Asn Val Leu Glu Asp Val Glu Val Asp Phe Lys Cys Thr Pro Ala Leu
165 170 175

Pro Lys Ser Ser Gln
180

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 500 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

CACTCATGTG AAATTTGTCG CAATTTTGGA AAAGATTGTG AGAGTGAGGA GGCAGAGGAA 60

TGTGCCTCTC CAGAAGATCA ATGTGGCACA GTGTTGCTGG AGATTTCATC AGCACCTATT 120

TCCTTCCGAT CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAACT TGAACACTTT 180

GATATAAATA TTGGACATGA TTCCTATGTG AGAGGAAGAA TCCACTGTTG TGATGAAGAA 240

AGGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC 300

CCTGGCATTC TTGGTGCATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA 360

ACCGAGACCA AATGCATTAA CCTTGCGGGA TTCAGAAAAG AAAGATATCC TGTAGACATC 420

GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAAGTTGAG CAATAGAACT 480

CACGAAGAAC GTAGAAATGA 500

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 500 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CGCTCATGTG AAATTTGTCA CAATTTTGGA AAAGTTTGCG AGAGTGAGGA GGCAGAGGAA 60

TGTGCCTCTC CAGAAGATCA ATGTGGCACA GTGTTGCTGG AGATTTCATC AGCACCTATT 120

TCCTTCCGAA CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAACT TGAACACTTT 180

GATATAAATA TTGGACATGA TTCCTATATC AGAGGAAGAA TCCACTGTTG TGATGAAGAA 240

AAGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC 300

CCTGGCATTC TTGGTGTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA 360

ACCGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAAAG AAAGATATCC TTTAGACATC 420

GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAG CAATAGAACT 480

CACGAAGAAC ACAGAAATGA 500

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 542 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

CTTGAGTGCG AGATTTGTAT TGGGCTGGGC CGGGAATGTA ACTCCTGGAC GAAAACCTGT 60

GATGCTAATC AAGATACTTG TGTTACCTTT CAAACTGAAG TGATAAGAGC CCCTGTGTCC 120

CTCTCTTTGA TTTCAAAATC CTGTGGTACT TCTGACACTT GCCATCTTAA CTACGTGGAG 180

ACGAGTCCAC ATAATGAACT AACGGTGAAG ACCAAAAGAA CCTGCTGTAC TGGGGAGGAA 240

TGTAAAACTC TGCCACCGCC TGTGCTTGGA TACAAAGTCA ACCCACCCAA CGGACTTCAG 300

TGTCCTGGAT GCCTTGGATT GTCCTCAAAA GAATGCACTG AACACCCGGT TTCCTGCCGG 360

GGATCTGAAA ACCAGTGTTT GTCTATAATT GGGAAGGAAT TTGGCCTTTT CTTCAGAGCA 420

TTGTCTTATA AAGGATGTGC TACGGAGAGT CTGTGCACTT TATTTGAGAA GAGGTTCTGG 480

AATGTTTTAG AGGATGTTGA GACTTCAAAT GCACGCCAGC CCTCCCAAAG TCTTCCCAGT 540

GA 542

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Met Lys Ser Leu Leu Phe Cys Cys Leu Phe Gly Thr Phe Leu Ala Thr
1 5 10 15

Gly Met Cys

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

ATGAAGTCCC TCTTATTCTG TTGCCTCTTT GGCACTTTCT TAGCTACAGG CATGTGT 57

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 183 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Arg Ser Cys Glu Thr Cys His Asn Phe Gly Lys Asp Cys Glu Ser Glu
1 5 10 15

Glu Ala Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly Thr Val Leu
20 25 30

Leu Glu Ile Ser Ser Ala Pro Ile Ser Phe Arg Ser Ile His Arg Asn
35 40 45

Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu His Phe Asp Ile Asn Ile
50 55 60

Gly His Asp Ser Tyr Val Arg Gly Arg Ile His Cys Cys Asn Glu Glu
65 70 75 80

Lys Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
85 90 95

Gly Tyr His Cys Pro Gly Ile Leu Gly Ala Phe Ser Val Asp Ser Ser
100 105 110

Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys Ile Asn Leu
115 120 125

Ala Gly Phe Arg Lys Glu Arg Tyr Pro Leu Asp Ile Ala Tyr Asn Ile
130 135 140

Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Ser Asn Arg Thr
145 150 155 160

His Glu Glu His Arg Asn Glu Leu Ile Lys Val Glu Cys Thr Asp Ala
165 170 175

Ser Lys Ile Thr Pro Ser Glu
180

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 183 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Arg Ser Cys Glu Ile Cys His Asn Phe Gly Lys Val Cys Glu Ser Glu
1 5 10 15

Glu Ala Glu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly Thr Val Leu
20 25 30

Leu Glu Ile Ser Ser Ala Pro Ile Ser Phe Arg Thr Ile His Arg Asn
35 40 45

Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu His Phe Asp Ile Asn Ile
50 55 60

Gly His Asp Ser Tyr Ile Arg Gly Arg Ile His Cys Cys Asp Glu Glu
65 70 75 80

Lys Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
85 90 95

Gly Tyr His Cys Pro Gly Ile Leu Gly Val Phe Ser Val Asp Ser Ser
100 105 110

Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys Ile Asn Leu
115 120 125

Ala Gly Phe Arg Lys Glu Arg Tyr Pro Leu Asp Ile Ala Tyr Asn Ile
130 135 140

Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Ser Asn Arg Thr
145 150 155 160

His Glu Glu His Arg Asn Glu Leu Ile Lys Val Glu Cys Thr Asp Ala
165 170 175

Ser Lys Ile Thr Pro Ser Glu

180

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 181 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

Leu Glu Cys Glu Ile Cys Ile Gly Leu Gly Arg Glu Cys Asn Ser Trp
1 5 10 15

Thr Lys Thr Cys Asp Ala Asn Gln Asp Thr Cys Val Thr Phe Gln Thr
20 25 30

Glu Val Ile Arg Ala Pro Val Ser Leu Ser Leu Ile Ser Lys Ser Cys
35 40 45

Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Val Glu Thr Ser Pro His
50 55 60

Asn Glu Leu Thr Val Lys Thr Lys Arg Thr Cys Cys Thr Gly Glu Glu
65 70 75 80

Cys Lys Thr Leu Pro Pro Pro Val Leu Gly Asp Lys Val Asn Pro Pro
85 90 95

Asn Gly Leu Gln Cys Pro Gly Cys Leu Gly Leu Ser Ser Lys Glu Cys
100 105 110

Thr Glu His Pro Val Ser Cys Arg Gly Ser Glu Asn Gln Cys Leu Ser
115 120 125

Ile Ile Gly Lys Glu Phe Gly Leu Phe Phe Arg Ala Leu Ser Tyr Lys
130 135 140

Gly Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Arg Phe Trp
145 150 155 160

Asn Val Leu Glu Asp Val Glu Val Asp Phe Lys Cys Ala Pro Ala Leu
165 170 175

Pro Lys Ser Ser Gln
180

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 182 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Leu Glu Cys Glu Phe Cys Phe Thr Pro Ala Leu Gln Cys Asp Asn Ser
1 5 10 15

Arg Thr Lys Thr Cys Asp Ala Asn Gln Asp Thr Cys Val Thr Ser Gln
20 25 30

Thr Glu Val Ile Arg Ala Pro Val Ser Leu Thr Phe Ile Ser Lys Ser
35 40 45

Cys Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Leu Glu Thr Ser Pro
50 55 60

His Asn Glu Leu Thr Val Lys Thr Lys Arg Thr Cys Cys Thr Gly Glu
65 70 75 80

Glu Cys Lys Thr Leu Pro Pro Pro Val Leu Gly Asp Lys Val Asn Pro
85 90 95

Pro Asn Gly Leu Gln Cys Pro Gly Cys Leu Gly Leu Ser Ser Lys Glu
100 105 110

Cys Thr Glu His Pro Val Ser Cys Arg Gly Ser Glu Asn Gln Cys Leu
115 120 125

Ser Ile Ile Gly Lys Glu Phe Gly Leu Phe Phe Arg Ala Leu Ser Tyr

130 135 140

Lys Gly Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Arg Phe
145 150 155 160

Trp Asn Val Leu Glu Asp Val Glu Val Asp Phe Lys Cys Thr Pro Ala
165 170 175

Leu Pro Lys Ser Ser Gln
180

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 500 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

CGCTCATGTG AAACTTGTCA CAATTTTGGA AAAGATTGCG AGAGTGAGGA GGCAGAGGAA 60

TGTGCCTCTC CAGAAGATCA ATGTGGCACA GTGTTGCTGG AGATTTCATC AGCACCTATT 120

TCCTTCCGAT CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAACT TGAACACTTT 180

GATATAAATA TTGGACATGA TTCCTATGTG AGAGGAAGAA TCCACTGTTG TAATGAAGAA 240

AAGTGCGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATATCACTGC 300

CCTGGCATCC TTGGTGCATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA 360

ACTGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAAAG AAAGATATCC CTTAGACATC 420

GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAG CAATAGAACT 480

CACGAAGAAC ACAGAAATGA 500

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 500 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

CGCTCATGTG AAATTTGTCA CAATTTTGGA AAAGTTTGTG AGAGTGAGGA GGCAGAGGAA 60

TGTGCCTCTC CAGAAGATCA ATGTGGCACA GTGTTGCTGG AGATTTCATC AGCACCTATT 120

TCCTTCCGAA CCATTCACAG GAACTGTTTC TCATCCAGCC TCTGCAAACT TGAACATTTT 180

GATATAAATA TTGGACATGA TTCCTATATC AGAGGAAGAA TCCACTGTTG TGATGAAGAA 240

AAGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATATCACTGC 300

CCTGGCATTC TTGGTGTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA 360

ACTGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAAAG AAAGATATCC TTTAGACATC 420

GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAG CAATAGAACT 480

CACGAAGAAC ACAGAAATGA 500

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 500 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

CTTGAGTGCG AGATTTGTAT TGGGCTGGGC CGGGAATGTA ACTCCTGGAC GAAAACCTGT 60

GATGCTAATC AAGATACTTG TGTTACCTTT CAAACTGAAG TGATAAGAGC CCCTGTGTCC 120

CTCTCTTTGA TTTCAAAATC CTGTGGTACT TCTGACACTT GCCATCTTAA CTACGTGGAG 180

ACGAGTCCAC ATAATGAACT AACGGTGAAG ACCAAAAGAA CCTGCTGTAC TGGGGAGGAA 240

TGTAAAACTC TGCCACCGCC TGTGCTTGGA GACAAAGTCA ACCCACCCAA CGGACTTCAG 300

TGTCCTGGAT GCCTTGGATT GTCCTCAAAA GAATGCACTG AACACCCGGT TTCCTGCCGG 360

GGATCTGAAA ACCAGTGTTT GTCTATAATT GGGAAGGAAT TTGGCCTTTT CTTCAGAGCA 420

TTGTCTTATA AAGGATGTGC TACGGAGAGT CTGTGCACTT TATTTGAGAA GAGGTTCTGG 480

AATGTTTTAG AGGATGTTGA 500

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 549 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

CTTGAGTGTG AGTTTTGTTT CACGCCAGCC CTGCAATGTG ATAACAGCAG GACGAAAACC 60

TGTGATGCTA ATCAAGATAC TTGTGTTACC TCTCAAACTG AAGTGATAAG AGCCCCTGTG 120

TCCCTCACTT TCATTTCAAA ATCCTGTGGT ACTTCTGACA CTTGCCATCT TAACTACTTG 180

GAGACGAGTC CACATAATGA ACTAACGGTG AAGACCAAAA GAACCTGCTG TACTGGGGAG 240

GAATGTAAAA CTCTGCCACC GCCTGTGCTT GGAGACAAAG TCAACCCACC CAACGGACTT 300

CAGTGTCCTG GATGCCTTGG ATTGTCCTCA AAAGAATGCA CTGAACACCC GGTTTCCTGC 360

CGGGGATCTG AAAACCAGTG TTTGTCTATA ATTGGGAAGG AATTTGGCCT TTTCTTCAGA 420

GCATTGTCTT ATAAAGGATG TGCTACGGAG AGTCTGTGCA CTTTATTTGA GAAGAGGTTC 480

TGGAATGTTT TAGAGGATGT TGAAGTAGAC TTCAAATGCA CGCCAGCCCT CCCAAAGTCT 540

TCCCAGTGA 549

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Met	Lys	Ser	Leu	Leu	Phe	Cys	Cys	Leu	Phe	Gly	Thr	Phe	Leu	Ala	Thr
1				5					10						15

Gly Met Cys

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Met	Lys	Ser	Leu	Leu	Phe	Cys	Cys	Leu	Phe	Gly	Thr	Phe	Leu	Ala	Thr
1				5					10						15

Gly Met Cys

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

ATGAAGTCCC TCTTATTCTG TTGCCTCTTT GGCACTTTCT TAGCTACAGG CATGTGT 57

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

ATGAAGTCCC TCTTATTCTG TTGCCTCTTT GGCACTTTCT TAGCTACAGG CATGTGT 57

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 183 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

Arg Ser Cys Glu Ile Cys His Asn Phe Gly Lys Val Cys Asp Asn Glu
1 5 10 15

Pro Ala Leu Glu Cys Ala Ser Pro Glu Asp Gln Cys Gly Thr Val Leu
20 25 30

Leu Glu Ile Ser Ser Ala Pro Ile Ser Phe Arg Thr Ile His Arg Asn
35 40 45

Cys Phe Ser Ser Ser Leu Cys Lys Leu Glu His Phe Asp Ile Asn Ile
50 55 60

Gly His Asp Ser Tyr Ile Arg Gly Arg Ile His Cys Cys Asp Glu Glu
65 70 75 80

Lys Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn
85 90 95

Gly Tyr His Cys Pro Gly Ile Leu Gly Val Phe Ser Val Asp Ser Ser
100 105 110

Glu His Glu Ala Ile Cys Arg Gly Thr Glu Thr Lys Cys Ile Asn Leu
115 120 125

Ala Gly Phe Arg Lys Glu Arg Thr Pro Leu Asp Ile Ala Tyr Asn Ile
130 135 140

Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Arg Leu Ser Asn Arg Thr
145 150 155 160

His Gly Gly His Arg Asn Glu Leu Ile Lys Val Glu Cys Thr Asp Ala
165 170 175

Pro Lys Ile Thr Pro Ser Glu
180

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 181 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

Leu Glu Cys Asp Ile Cys Ile Gly Leu Gly Arg Glu Cys Asn Thr Trp
1 5 10 15

Thr Lys Thr Cys Asp Ala Asn Gln Asp Ala Cys Val Thr Phe Gln Thr
20 25 30

Glu Val Ile Arg Ala Pro Val Ser Leu Ser Leu Ile Ser Lys Ser Cys
35 40 45

Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Leu Glu Thr Ser Pro His
50 55 60

Asn Glu Leu Thr Val Lys Thr Lys Arg Thr Cys Cys Thr Gly Glu Glu

65 70 75 80

Cys Lys Thr Leu Pro Pro Pro Val Leu Gly Asp Lys Val Ser Pro Pro
85 90 95

Asn Gly Leu Gln Cys Pro Gly Cys Phe Gly Leu Ser Ser Lys Glu Cys
100 105 110

Thr Glu His Pro Val Ser Cys Arg Gly Ser Glu Asn Gln Cys Leu Ser
115 120 125

Ile Ile Gly Lys Glu Phe Gly Leu Phe Phe Arg Ala Leu Ser Tyr Lys
130 135 140

Gly Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Lys Phe Trp
145 150 155 160

Asn Val Leu Glu Asp Val Glu Val Asp Phe Lys Cys Thr Pro Ala Leu
165 170 175

Pro Lys Ser Ser Gln
180

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 181 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Leu Glu Cys Asp Ile Cys Phe Gly Leu Gly Arg Lys Cys Asn Thr Trp
1 5 10 15

Thr Lys Thr Cys Asp Ala Asn Gln Asp Ala Cys Val Thr Phe Gln Thr
20 25 30

Glu Val Ile Arg Ala Pro Val Ser Leu Ser Leu Ile Ser Lys Ser Cys
35 40 45

Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Leu Glu Thr Ser Pro His
50 55 60

Asn Glu Leu Thr Val Lys Thr Lys Arg Thr Cys Cys Thr Gly Glu Glu
65 70 75 80

Cys Lys Thr Leu Pro Pro Pro Val Leu Gly Asp Lys Val Ser Pro Pro
85 90 95

Asn Gly Leu Gln Cys Pro Gly Cys Phe Gly Leu Ser Ser Lys Glu Cys
100 105 110

Thr Glu His Pro Val Ser Cys Arg Gly Ser Glu Asn Gln Cys Leu Ser
115 120 125

Leu Ile Gly Lys Glu Phe Gly Phe Phe Phe Arg Ala Leu Ser Tyr Lys
130 135 140

Gly Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Lys Phe Trp
145 150 155 160

Asn Val Leu Glu Glu Val Glu Val Asp Phe Lys Cys Thr Pro Ala Leu
165 170 175

Pro Lys Ser Ser Gln
180

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 500 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

CGCTCATGTG AAATTTGTCA CAATTTTGGA AAAGTTTGCG ACAATGAGCC GGCATTGGAA 60

TGTGCCTCTC CAGAAGATCA ATGTGGCACA GTGTTGCTGG AGATTTCATC GGCACCTATT 120

TCCTTCCGAA CCATTCATAG GAACTGTTTC TCATCCAGCC TCTGCAAACT TGAACACTTT 180

GATATAAATA TTGGACATGA TTCCTATATC AGAGGAAGAA TCCACTGTTG TGATGAAGAA 240

AAGTGTGAAG CACAGCAATT TCCTGGACTG CCCCTCTCCT TTCCAAATGG ATACCACTGC 300

CCTGGCATTC TTGGTGTATT CTCAGTGGAC AGCTCTGAAC ATGAAGCTAT TTGCAGAGGA 360

ACTGAAACCA AATGCATTAA CCTTGCGGGA TTCAGAAAAG AAAGAACTCC TTTAGACATC 420

GCTTATAATA TCAAAGGTTG CACTTCTTCT TGTCCAGAAC TGAGGTTGAG CAATAGAACT 480

CACGGAGGAC ATAGAAATGA 500

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 500 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

CTTGAGTGTG ATATTTGTAT TGGGCTGGGC CGGGAATGTA ACACCTGGAC GAAAACCTGT 60

GACGCTAATC AAGATGCTTG TGTTACCTTT CAAACTGAAG TGATAAGAGC CCCTGTGTCC 120

CTCTCTTTGA TTTCAAAATC CTGTGGTACT TCTGACACTT GCCATCTTAA CTACCTGGAG 180

ACGAGTCCAC ATAATGAACT AACGGTGAAG ACCAAAAGAA CCTGCTGTAC TGGGGAGGAA 240

TGTAAAACTC TGCCACCGCC TGTGCTTGGA GACAAAGTCA GCCCACCCAA CGGACTTCAG 300

TGTCCTGGAT GCTTTGGATT GTCCTCAAAA GAATGCACTG AACACCCGGT TCCTGCCGG 360

GGATCTGAAA ACCAGTGCTT GTCCATAATT GGGAAGGAAT TTGGCCTTTT CTTCAGAGCA 420

TTGTCTTATA AAGGATGTGC TACGGAGAGT CTGTGCACTT TATTTGAGAA GAAGTTCTGG 480

AATGTTTTAG AGGATGTTGA 500

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 546 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

CTTGAGTGTG ATATTTGTTT TGGGCTGGGC CGGAAATGTA ACACCTGGAC GAAAACCTGT 60

GATGCTAATC AAGATGCTTG TGTTACTTTT CAAACTGAAG TGATAAGAGC CCCTGTGTCC 120

CTCTCTTTGA TTTCAAAATC CTGTGGTACT TCTGACACTT GCCATCTTAA CTACCTGGAG 180

ACGAGTCCAC ATAATGAACT AACGGTGAAG ACCAAAAGAA CCTGCTGTAC TGGGGAGGAA 240

TGTAAAACTC TGCCACCGCC TGTGCTTGGA GACAAAGTCA GCCCACCCAA CGGACTTCAG 300

TGTCCTGGAT GCTTTGGATT GTCCTCAAAA GAATGCACTG AACACCCGGT TTCCTGCCGG 360

GGATCTGAAA ACCAGTGTCT GTCTCTAATT GGGAAGGAAT TTGGCTTTTT CTTCAGAGCA 420

TTGTCTTATA AAGGATGTGC TACGGAGAGT CTGTGCACTC TATTTGAGAA GAAGTTCTGG 480

AATGTTTTAG AGGAAGTTGA AGTAGACTTC AAATGCACCC CAGCCCTCCC AAAGTCTTCC 540

CAGTGA 546

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Met Lys Ser Leu Gln Ile Ile Cys Leu Leu Phe Val Leu Val Ala Arg
1 5 10 15

Gly Ser Cys

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Met Lys Ser Leu Leu Phe Cys Cys Leu Phe Gly Thr Phe Leu Ala Thr
1 5 10 15

Gly Met Cys

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Met Lys Ser Leu Leu Phe Cys Cys Leu Phe Gly Thr Phe Leu Ala Thr
1 5 10 15

Gly Met Cys

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

ATGAAATCCC TACAGATCAT CTGTCTTCTT TTCGTTTTGG TAGCCAGAGG AAGCTGT 57

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

ATGAAGTCCC TCTTATTCTG TTGCCTCTTT GGCACTTTCT TAGCTACAGG CATGTGT 57

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

ATGAAGTCCC TCTTATTCTG TTGCCTCTTT GGCACTTTCT TAGCCACAGG CATGTGT 57

CLAIMS:

1. An isolated molecule capable of inhibiting two or more types of phospholipase enzymes.

2. The isolated molecule according to claim 1 wherein the phospholipase enzyme is a PLA_2.

3. The isolated molecule according to claim 2 wherein the types of phospholipase enzymes are PLA_2 Type I, Type II and/or Type III.

4. The isolated molecule according to any one of claims 1 to 3 derivable from the serum of a venomous animal.

5. The isolated molecule according to claim 4 wherein the venomous animal is a snake.

6. The isolated molecule according to claim 5 wherein the snake is selected from *Notechis scutatus, Notechis ater, Oxyuranus scutellatus, Oxyuranus microlepidotus* and *Pseudonaja textilis* or a relative thereof.

7. The isolated molecule according to claim 6 in sequencably pure and/or substantially homogeneous form.

8. The molecule according to claim 7 when associated with a carrier molecule.

9. The isolated molecule according to claim 7 having an amino acid sequence comprising one or more of SEQ ID NOs: 1-4 or 9-12 or an amino acid sequence having at least about 40% similarity thereto.

10. The isolated molecule according to claim 7 having an amino acid sequence comprising one or more of SEQ ID NOs: 17-38 or 43-45 or an amino acid sequence having at least

about 40% similarity thereto.

11. The isolated molecule according to claim 7 having an amino acid sequence comprising one or more of SEQ ID NOs: 51-53 or 57-59 or an amino acid sequence having at least about 40% similarity thereto.

12. The isolated molecule according to claim 7 having an amino acid sequence comprising one or more of SEQ ID NOs: 63-66 or 71-74 or an amino acid sequence having at least about 40% similarity thereto.

13. The isolated molecule according to claim 7 having an amino acid sequence comprising one or more of SEQ ID NOs: 79-81 or 85-87 or an amino acid sequence having at least about 40% similarity thereto.

14. A PLA_2 inhibitor $\alpha_m\beta_n$ wherein α is an α-chain of a PLA_2 inhibitor; β is a β-chain of a PLA_2 inhibitor; m is an integer from 0 to 10; n is an integer from 0 to 10 with proviso that if m and n are not 0, then m>n and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α comprises an amino acid sequence selected from SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 and 85 or an amino acid sequence having at least about 40% similarity to one or more of said sequences and β comprises an amino acid sequence selected from SEQ ID NOs: 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 and 87 or an amino acid sequence having at least about 40% similarity to one or more of said sequences.

15. The isolated molecule according to claim 1 when used to inhibit a phospholipase enzyme.

16. A composition useful for the inhibition of phospholipase enzyme activity comprising a molecule according to any one of claims 1 to 14 and a pharmaceutically acceptable carrier and/or diluent.

17. An isolated nucleic acid molecule which comprises a sequence of nucleotides encoding or complementary to a sequence encoding a polypeptide capable of inhibiting two or more types of phospholipase enzymes.

18. An isolated nucleic acid molecule according to claim 17 wherein the phospholipase enzyme is a PLA_2.

19. An isolated nucleic acid molecule according to claim 18 wherein the types of phospholipase enzymes are PLA_2 Type I, Type II and/or Type III.

20. An isolated nucleic acid molecule according to claim 18 or 19 wherein the PLA_2 is derivable from the serum of a venomous animal.

21. An isolated nucleic acid molecule according to claim 20 wherein the venomous animal is a snake.

22. An isolated nucleic acid molecule according to claim 21 wherein the snake is selected from *Notechis scutatus, Notechis ater, Oxyuranus scutellatus, Oxyuranus microlepidotus* and *Pseudonaja textilis* or a relative thereof.

23. An isolated nucleic acid molecule according to claim 22 encoding an amino acid sequence comprising one or more of SEQ ID NOs: 1-4 or 9-12 or an amino acid sequence having at least about 40% similarity thereto.

24. An isolated nucleic acid molecule according to claim 22 encoding an amino acid sequence comprising one or more of SEQ ID NOs: 17-38 or 43-45 or an amino acid sequence having at least about 40% similarity thereto.

25. An isolated nucleic acid molecule according to claim 22 encoding an amino acid sequence comprising one or more of SEQ ID NOs: 51-53 or 57-59 or an amino acid sequence having at least about 40% similarity thereto.

26. An isolated nucleic acid molecule according to claim 22 encoding an amino acid sequence comprising one or more of SEQ ID NOs: 63-66 or 71-74 or an amino acid sequence having at least about 40% similarity thereto.

27. An isolated nucleic acid molecule according to claim 22 encoding an amino acid sequence comprising one or more of SEQ ID NOs: 79-81 or 85-87 or an amino acid sequence having at least about 40% similarity thereto.

28. An isolated nucleic acid molecule according to claim 22 comprising one or more of SEQ ID NOs: 5-8 or 13-16 or a nucleotide sequence having at least 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to one or more sequences under low stringency conditions at 42°C.

29. An isolated nucleic acid molecule according to claim 22 comprising one or more of SEQ ID NOs: 39-42 or 47-50 or a nucleotide sequence having at least 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to one or more sequences under low stringency conditions at 42°C.

30. An isolated nucleic acid molecule according to claim 22 comprising one or more of SEQ ID NOs: 54-56 or 60-62 or a nucleotide sequence having at least 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to one or more sequences under low stringency conditions at 42°C.

31. An isolated nucleic acid molecule according to claim 22 comprising one or more of SEQ ID NOs: 67-70 or 75-78 or a nucleotide sequence having at least 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to one or more sequences under low stringency conditions at 42°C.

32. An isolated nucleic acid molecule according to claim 22 comprising one or more of SEQ ID NOs: 82-84 or 88-90 or a nucleotide sequence having at least 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to one or more

sequences under low stringency conditions at 42°C.

33. A nucleic acid molecule encoding a PLA_2 inhibitor having the structure:

$$\alpha_m\beta_n$$

wherein

α is an α-chain of a PLA_2 inhibitor;

β is a β-chain of a PLA_2 inhibitor; m is an integer from 0 to 10;

n is an integer from 0 to 10 with proviso that if m and n are not 0, then m>n and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α comprises an amino acid sequence selected from SEQ ID NOs. 1-3, 9-11, 17-23, 35-37, 43-45, 51, 52, 57, 58, 63, 64, 71, 72, 79 and 85 or an amino acid sequence having at least about 40% similarity to one or more of said sequences and β comprises an amino acid sequence selected from SEQ ID NOs: 4, 12, 24-34, 38, 46, 53, 59, 65, 66, 73, 74, 80, 81, 86 and 87 or an amino acid sequence having at least about 40% similarity to one or more of said sequences.

34. A nucleic acid molecule encoding a PLA_2 inhibitor having the structure:

$$\alpha_m\beta_n$$

wherein

α is an α-chain of a PLA_2 inhibitor;

β is a β-chain of a PLA_2 inhibitor; m is an integer from 0 to 10;

n is an integer from 0 to 10 with proviso that if m and n are not 0, then m>n and if m is 0, n cannot be 0 or if n is 0, m cannot be 0 and wherein α is encoded by an nucleotide sequence selected from SEQ ID NOs. 5-7, 13-15, 39-41, 47-49, 54, 55, 60, 61, 67, 68, 75, 76, 82 and 88 or a nucleotide sequence having at least about 40% similarity to one or more of said sequences or a nucleotide sequence capable of hybridizing to one or more of said sequences under low stringency conditions at 42°C and β is encoded by a nucleotide sequence selected from SEQ ID NOs: 8, 16, 42, 50, 56, 62, 69, 70, 77, 78, 83, 84, 89, 90 or a nucleotide

sequence capable of hybridizing to one or more of said sequences under low stringency conditions at 42°C.

35. A method of treatment of the phospholipase-related symptom(s) of rheumatoid arthritis, osteoarthritis, asthma, allergic reaction, psoriasis, multiple organ failure, acute pancreatitis, acute lung failure, septic shock, adult respiratory distress syndrome or the toxic effects of toxins in a human or animal subject, said method comprising administering an isolated molecule according to any one of claims 1 to 14 to a subject for a time and under conditions sufficient to partially or completely inhibit the activity of a phospholipase enzyme producing said symptom(s).

36. A method of isolating a PLA_2 inhibitor protein from snake blood, serum or other blood product at least comprising the steps of:

(i) preparing a serum sample from clotted blood; and

(ii) subjecting the serum to ion-exchange chromatography.

37. The method according to paragraph 36 wherein the PLA_2 inhibitor protein is selected from NSI, NAI, OSI, OMI and PTI.

38. An isolated polypeptide capable of inhibiting two or more of PLA_2 Type I, II and/or III wherein said polypeptide has an alpha chain comprising the following amino acid sequence:

Xaa Ser Cys Glu Xaa Cys Xaa Asn Xaa Gly Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Glu Cys Ala Ser Xaa Glu Asp Gln Cys Gly Thr Val Leu Xaa Glu Xaa Ser Xaa Ala Pro Ile Ser Xaa Arg Xaa Ile His Arg Xaa Cys Phe Ser Ser Ser Xaa Cys Lys Leu Glu Xaa Phe Asp Ile Asn Ile Gly His Asp Ser Xaa Xaa Arg Gly Arg Ile His Cys Cys Xaa Glu Xaa Xaa Cys Glu Ala Gln Gln Phe Pro Gly Leu Pro Leu Ser Phe Pro Asn Gly Tyr His Cys Pro Gly Ile Xaa Gly Xaa Phe Ser Val Asp Ser Ser Glu His Glu Ala Ile Cys Arg Gly Xaa Glu Thr Lys Cys Ile Xaa Xaa Ala Gly Phe Arg Xaa Glu Arg Xaa Xaa Xaa Asp Xaa Xaa Tyr Asn Ile Lys Gly Cys Thr Ser Ser Cys Pro Glu Leu Xaa Leu Xaa Asn Arg Thr His Xaa Xaa Xaa Xaa Asn Xaa Leu Ile

Xaa Xaa Glu Cys Thr Xaa Ala Xaa Lys Xaa Xaa Pro Ser Glu.

39. An isolated polypeptide of claim 38 encoded by the following nucleotide sequence:

CNCTCATGTGAAANTTGTCNCAATTTNGGAANAGNNTGNNANNNTGNNNNGNCA
NNGGAATGTGCNTCTNCAGAAGATCAATGTGGCACNGTGTTGNTGGAGNTTTCA
NCNGCACCTATTTCCNNCCGANCCATTCANAGGAANTGTTTCTCATCCAGCNTCT
GCAAACTNGAACNNTTTGATATAAATATTGGACATGATTCCTNTNTNAGAGGAA
GAATCCACTGTTGTNATGAAGNAANGTGNGAAGCACAGCAATTTCCTGGACTGC
CCCTCTCCTTTCCAAATGGATANCACTGCCCTGGNATNNTTGGTNNATTCTCAGT
GGACAGNTCTGAACATGAAGCTATTTGCAGAGGAANNGANACCAAATGCATTAA
NNTTGCGGGATTCAGAANNGAAAGANNTNNNNNAGACATNGNTTATAATATCAA
AGGTTGCACTTCTTCTTGTCCAGAACTGANGTTGANNNATAG

or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C.

40. An isolated nucleic acid molecule comprising the nucleotide sequence:

CNCTCATGTGAAANTTGTCNCAATTTNGGAANAGNNTGNNANNNTGNNNNGNCA
NNGGAATGTGCNTCTNCAGAAGATCAATGTGGCACNGTGTTGNTGGAGNTTTCA
NCNGCACCTATTTCCNNCCGANCCATTCANAGGAANTGTTTCTCATCCAGCNTCT
GCAAACTNGAACNNTTTGATATAAATATTGGACATGATTCCTNTNTNAGAGGAA
GAATCCACTGTTGTNATGAAGNAANGTGNGAAGCACAGCAATTTCCTGGACTGC
CCCTCTCCTTTCCAAATGGATANCACTGCCCTGGNATNNTTGGTNNATTCTCAGT
GGACAGNTCTGAACATGAAGCTATTTGCAGAGGAANNGANACCAAATGCATTAA
NNTTGCGGGATTCAGAANNGAAAGANNTNNNNNAGACATNGNTTATAATATCAA
AGGTTGCACTTCTTCTTGTCCAGAACTGANGTTGANNNATAG

or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C.

41. An isolated polypeptide capable of inhibiting two or more of PLA_2 Type I, II and/or III wherein said polypeptide has a beta chain comprising the following amino acid sequence:

Leu Glu Cys Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Asn Xaa Xaa Thr Lys Thr Cys Asp Ala Asn Gln Asp Xaa Cys Val Thr Xaa Gln Thr Glu Val Ile Arg Ala Pro Val Ser Leu Xaa Xaa Ile Ser Lys Ser Cys Gly Thr Ser Asp Thr Cys His Leu Asn Tyr Xaa Glu Thr Ser Pro His Asn Glu Leu Thr Val Lys Thr Lys Arg Thr Cys Cys Thr Gly Glu Glu Cys Lys Thr Leu Pro Pro Pro Val Leu Gly Xaa Lys Val Xaa Pro Pro Asn Gly Leu Gln Cys Pro Gly Cys Xaa Gly Leu Ser Ser Lys Glu Cys Thr Glu His Xaa Val Ser Cys Arg Gly Ser Glu Asn Gln Cys Leu Ser Xaa Ile Gly Lys Glu Phe Gly Xaa Phe Phe Arg Ala Leu Ser Tyr Lys Gly Cys Ala Thr Glu Ser Leu Cys Thr Leu Phe Glu Lys Xaa Phe Trp Asn Val Leu Glu Xaa Val Glu Val Asp Phe Lys Cys Xaa Pro Ala Leu Pro Lys Ser Ser Gln.

42. An isolated polypeptide of claim 38 encoded by the following nucleotide sequence:

CTTGAGTGNGANNTTTGTNTNNNGCNNGNCCNGNAATGTNNNAACNNCGGACG
AAAACCTGTGANGCTAATCAAGATNCTTGTGTTACNTNTCAAACTGAAGTGATA
AGAGCCCCTGTGTCCCTCNCTTTNATNTCAAAATCCTGTGGTACTTCTGACACTT
GCCATCTTAACTACNTGGAGACGAGTCCACATAATGAACTAACNGTGAAGACCA
AAAGAACCTGCTGTACTGGGGAGGAATGTAAAACTCTGCCACCGCCTGTGCTTG
GANACAAAGTCANCCCACCCAACGGACTTCAGTGTCCTGGATGCNTTGGATTGT
CCTCAAAAGAATGCACTGAACACCNGGTTTCCTGCCGGGGATCTGAAAACCAGT
GNNTGTCNNTAATTGGGAANGAATTTGGCNTTTTCTTCAGAGCATTGTCTTATAA
AGGATGTGCTACGGAGAGTCTGTGCACTNTATTTGAGAAGANGTTCTGGAATGT
TTTAGAGGANGTTGAAGTAGACTTCAAATGCNCNCCNGCCCTCCCAAAGTCTTCC
CAGNNN

or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C.

43. An isolated nucleic acid molecule comprising the nucleotide sequence:

CTTGAGTGNGANNTTTGTNTNNNGCNNGNCCNGNAATGTNNNAACNNCGGACG
AAAACCTGTGANGCTAATCAAGATNCTTGTGTTACNTNTCAAACTGAAGTGATA
AGAGCCCCTGTGTCCCTCNCTTTNATNTCAAAATCCTGTGGTACTTCTGACACTT
GCCATCTTAACTACNTGGAGACGAGTCCACATAATGAACTAACNGTGAAGACCA
AAAGAACCTGCTGTACTGGGGAGGAATGTAAAACTCTGCCACCGCCTGTGCTTG
GANACAAAGTCANCCCACCCAACGGACTTCAGTGTCCTGGATGCNTTGGATTGT
CCTCAAAAGAATGCACTGAACACCNGGTTTCCTGCCGGGGATCTGAAAACCAGT
GNNTGTCNNTAATTGGGAANGAATTTGGCNTTTTCTTCAGAGCATTGTCTTATAA
AGGATGTGCTACGGAGAGTCTGTGCACTNTATTTGAGAAGANGTTCTGGAATGT
TTTAGAGGANGTTGAAGTAGACTTCAAATGCNCNCCNGCCCTCCCAAAGTCTTCC
CAGNNN

or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C.

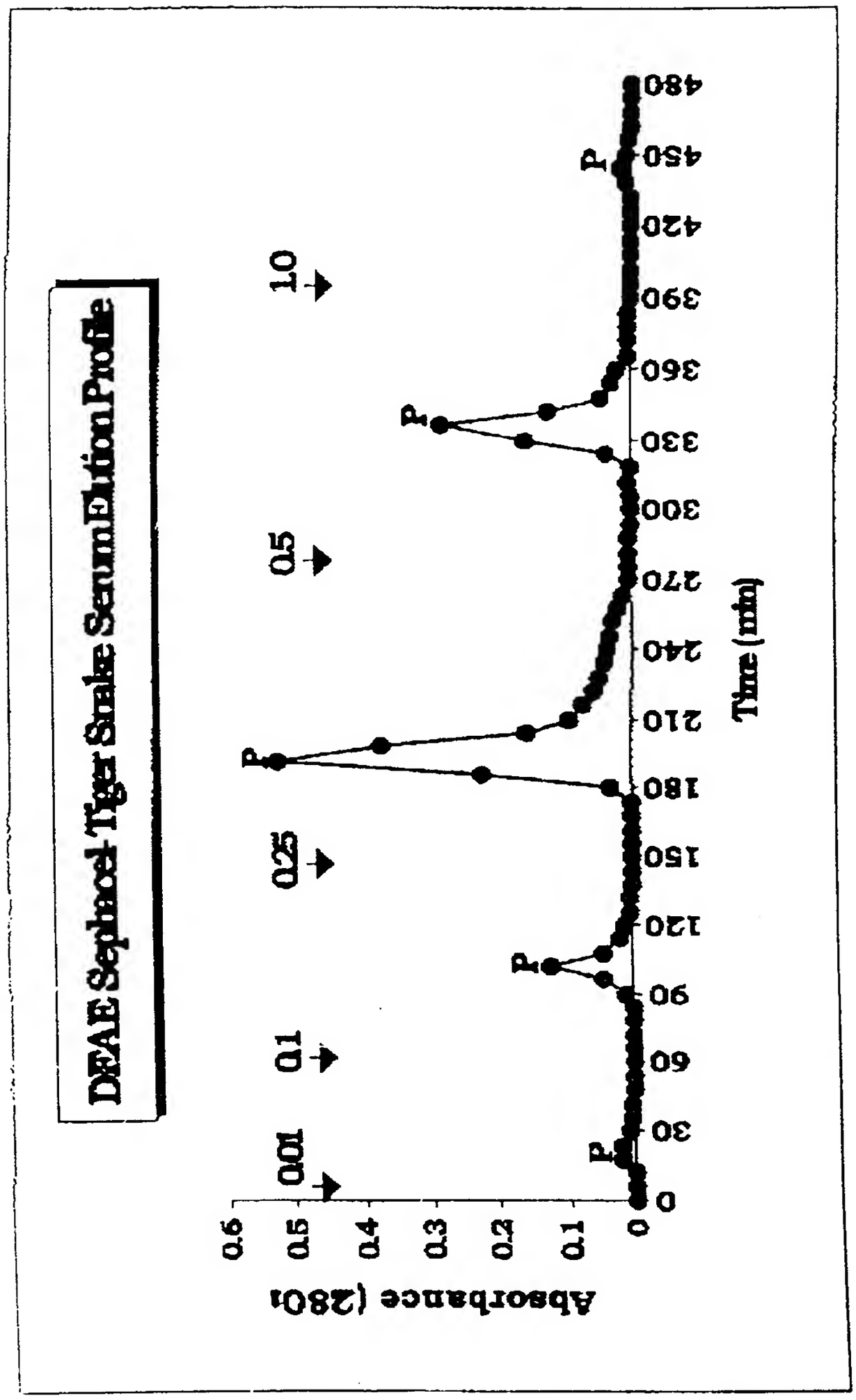
DEAE Sephacel Tiger Snake Serum Elution Profile
Absorbance (280)
0.6
0.5
0.4
0.3
0.2
0.1
0
0.01
0.1
0.25
0.5
1.0
P1
P2
P3
P4
P5
0
30
60
90
120
150
180
210
240
270
300
330
360
390
420
450
480
Time (min)

FIGURE 1A

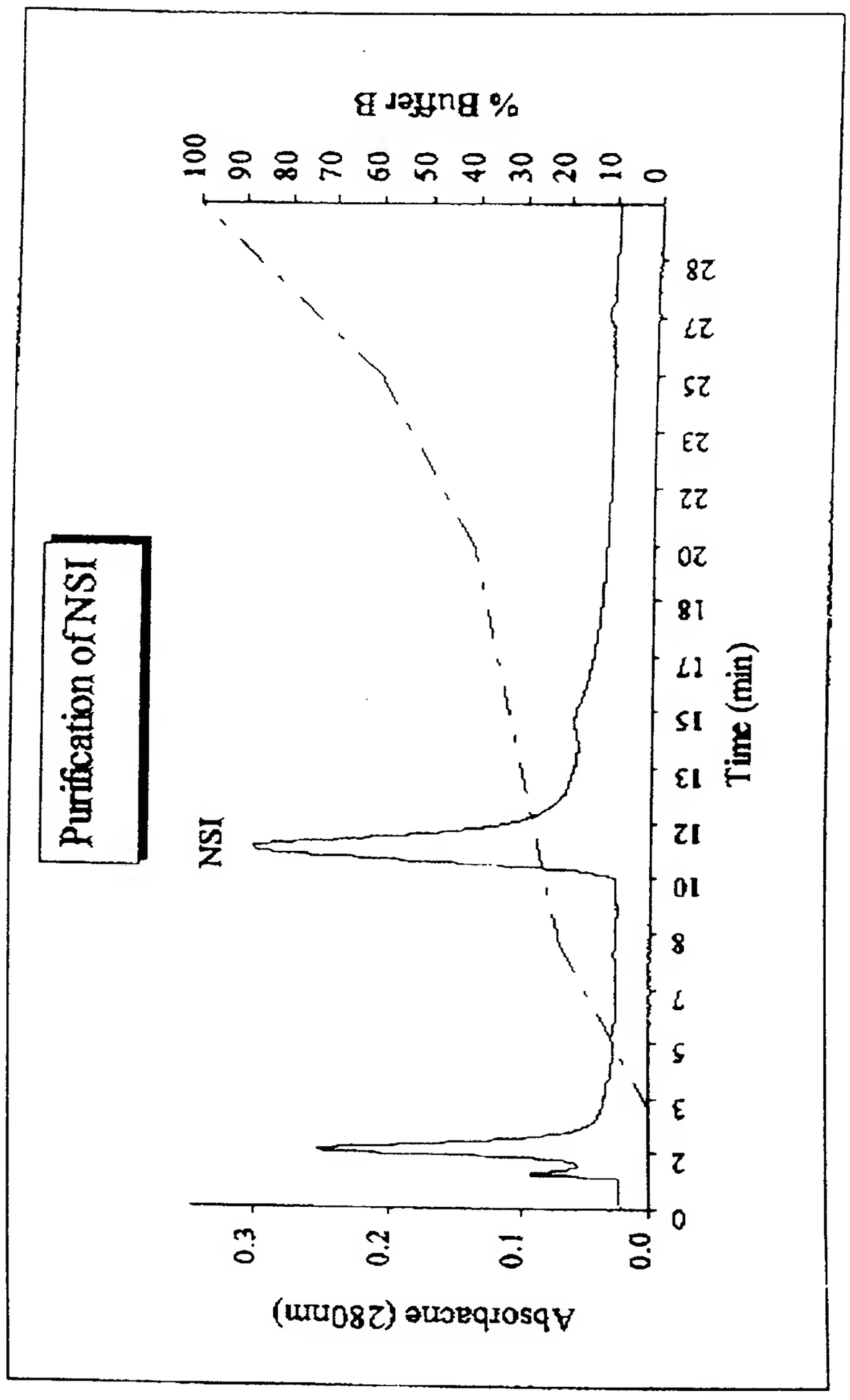
% Buffer B
100
90
80
70
60
50
40
30
20
10
0
28
27
25
23
22
20
18
17
15
13
12
10
8
7
5
3
2
0
Time (min)
Purification of NSI
NSI
0.3
0.2
0.1
0.0
Absorbacne (280nm)

FIGURE 1B

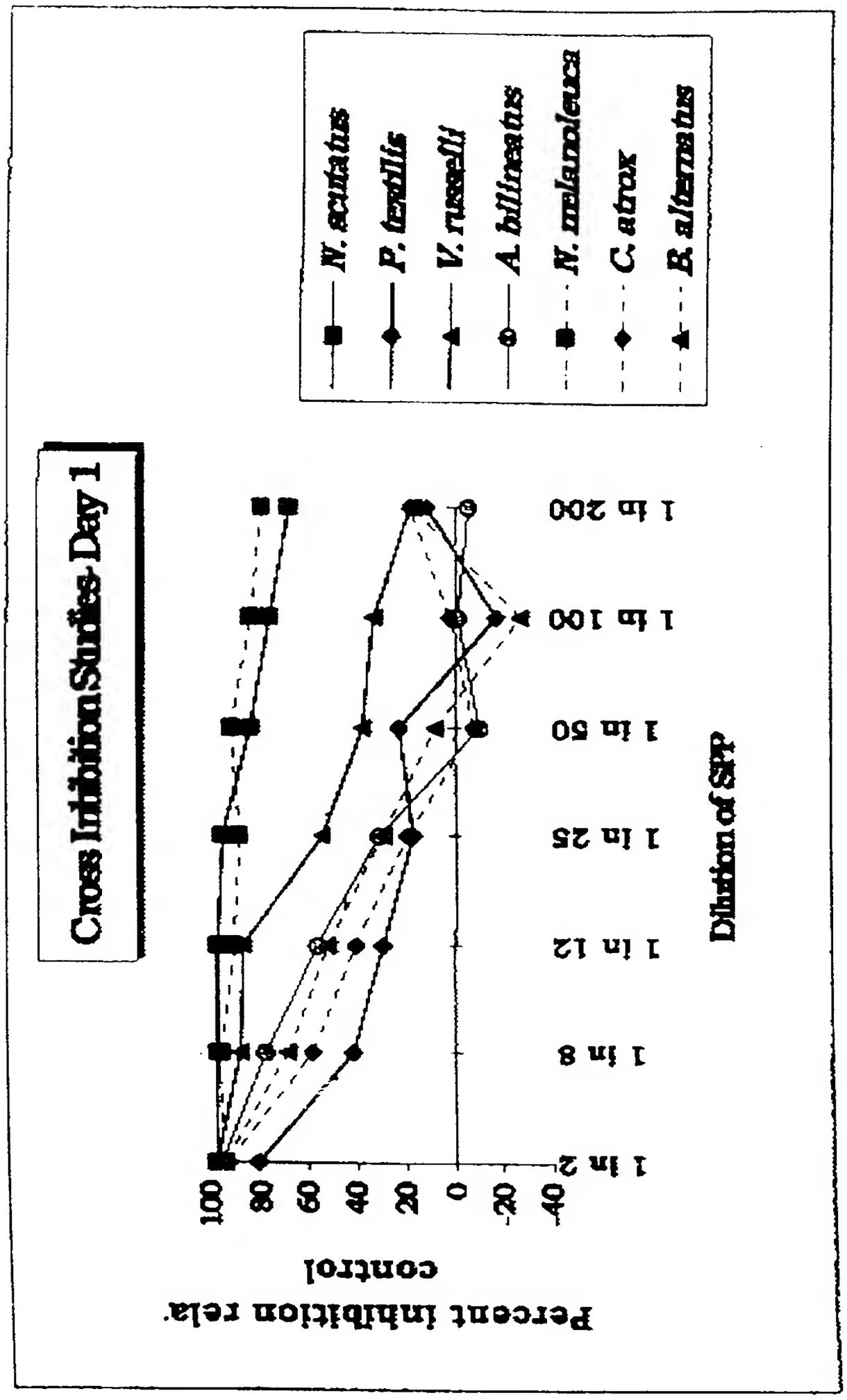
N. scutatus
P. textilis
V. russelli
A. hilliseatus
N. melanoleuca
C. atrox
B. alternatus
Cross Inhibition Studies Day 1
1 in 200
1 in 100
1 in 50
1 in 25
1 in 12
1 in 8
1 in 2
Dilution of SIP
100
80
60
40
20
0
20
40
Percent inhibition rela.
control

FIGURE 2A

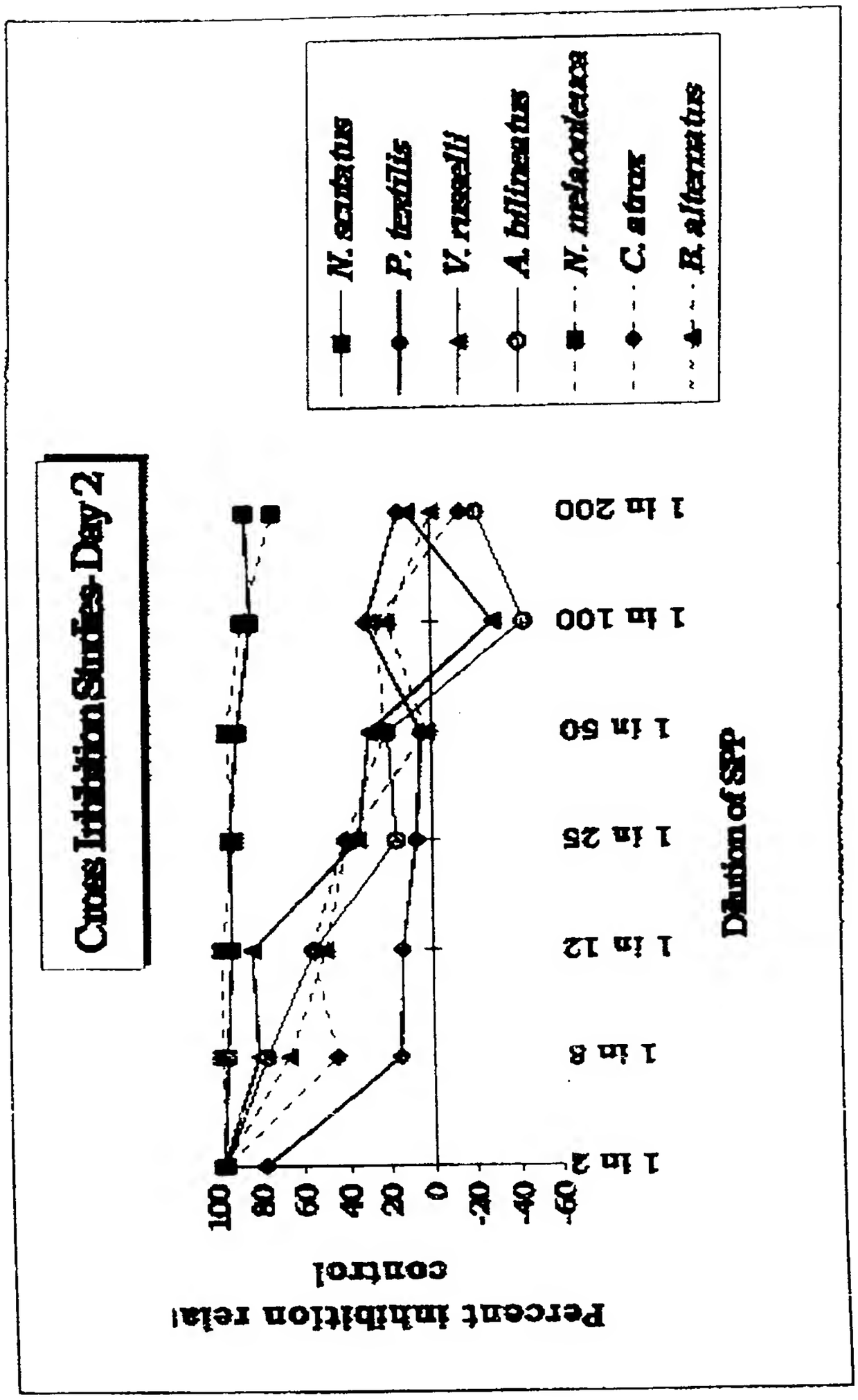
N. scutatus
P. textilis
V. russelli
A. bilineatus
N. melanoleuca
C. atrox
B. alternatus
Cross Inhibition Studies- Day 2
1 in 200
1 in 100
1 in 50
1 in 25
1 in 12
1 in 8
1 in 2
Dilution of SIP
100
80
60
40
20
0
20
40
60
Percent inhibition rela
control

FIGURE 2B

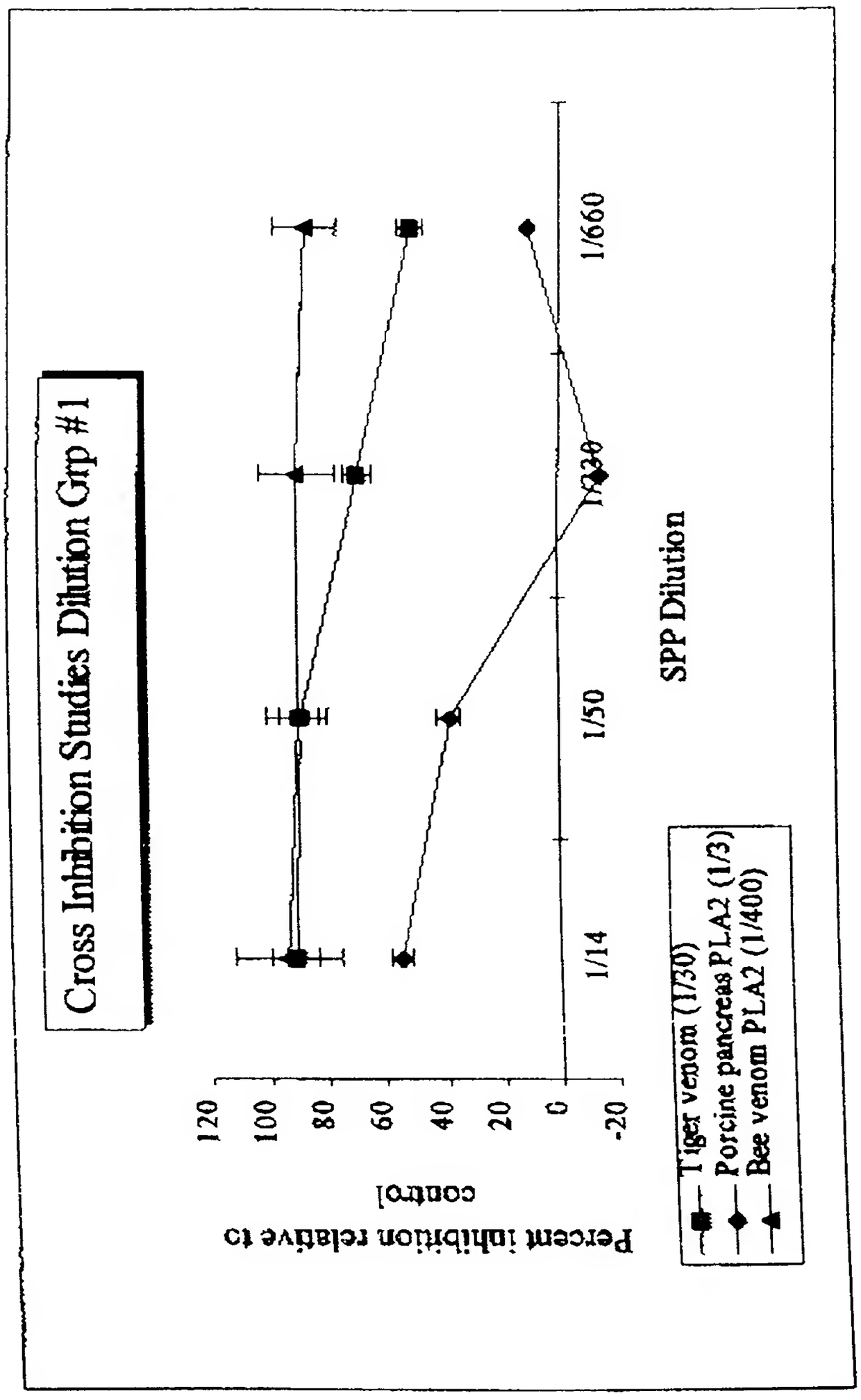
Cross Inhibition Studies Dilution Grp #1
Percent inhibition relative to control
120
100
80
60
40
20
0
-20
1/14
1/50
1/230
1/660
SPP Dilution
Tiger venom (1/30)
Porcine pancreas PLA2 (1/3)
Bee venom PLA2 (1/400)

FIGURE 3A

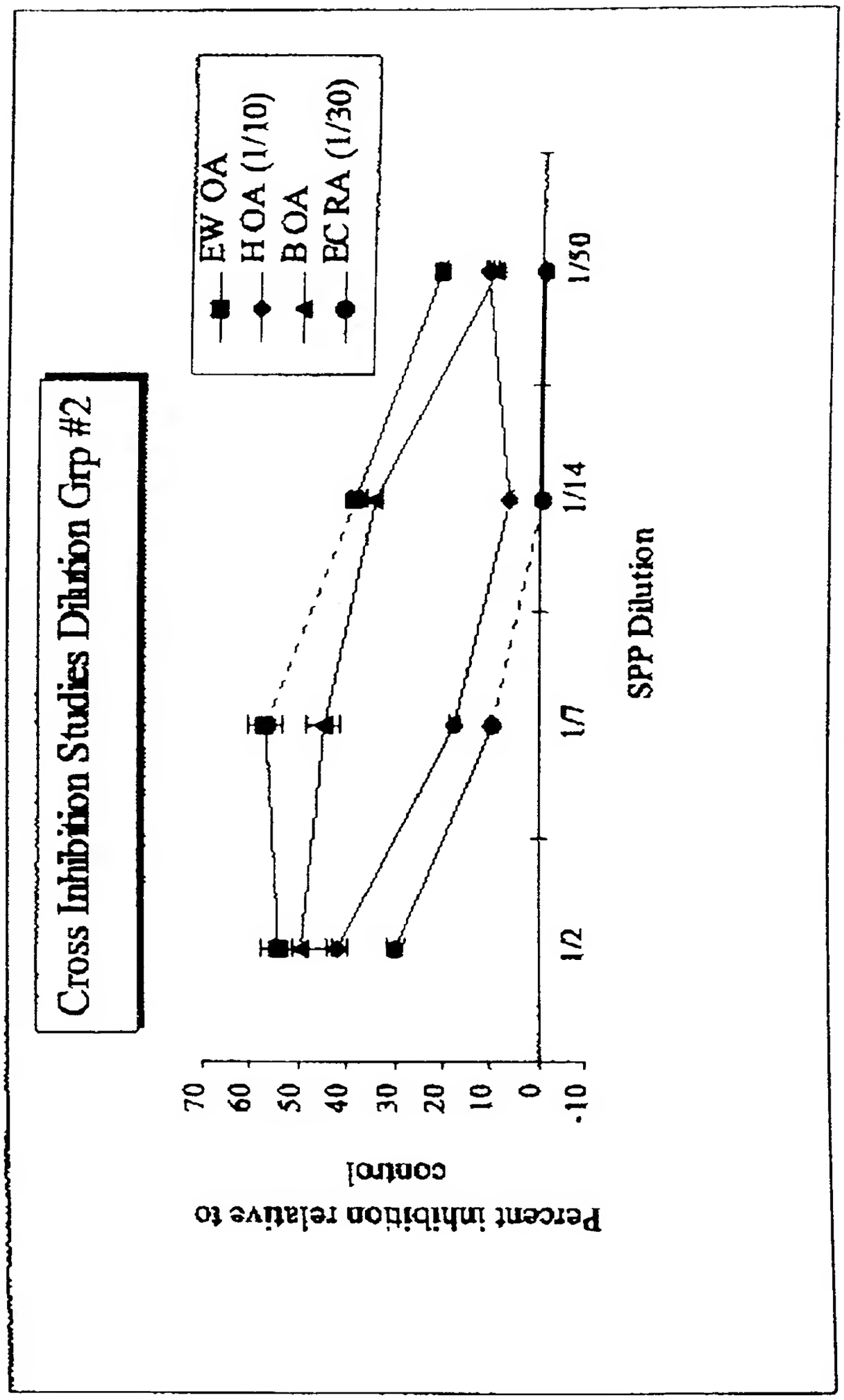
Cross Inhibition Studies Dilution Grp #2
EW OA
H OA (1/10)
B OA
EC RA (1/30)
1/50
1/14
1/7
1/2
SPP Dilution
70
60
50
40
30
20
10
0
-10
Percent inhibition relative to control

FIGURE 3B

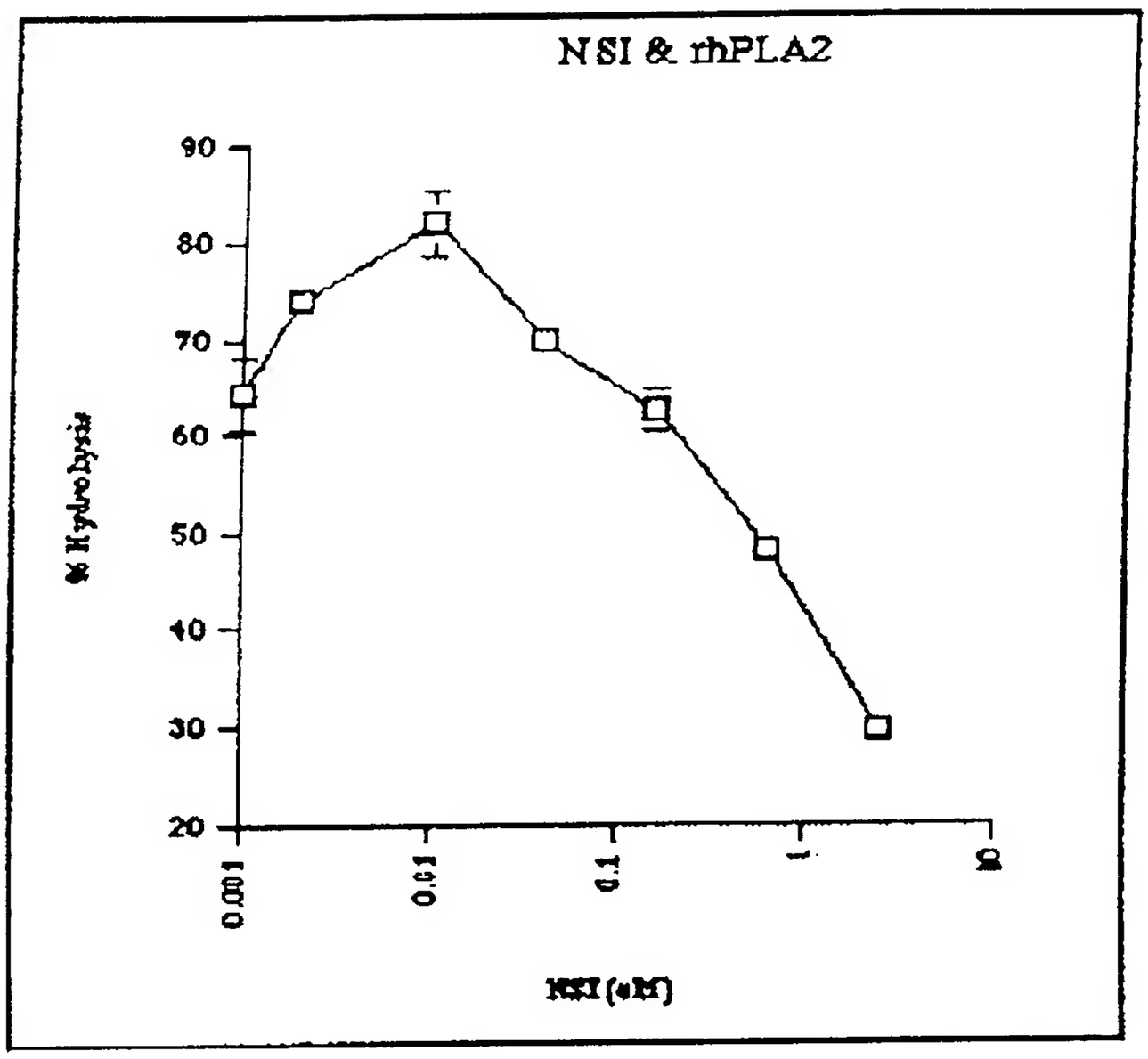

FIGURE 4

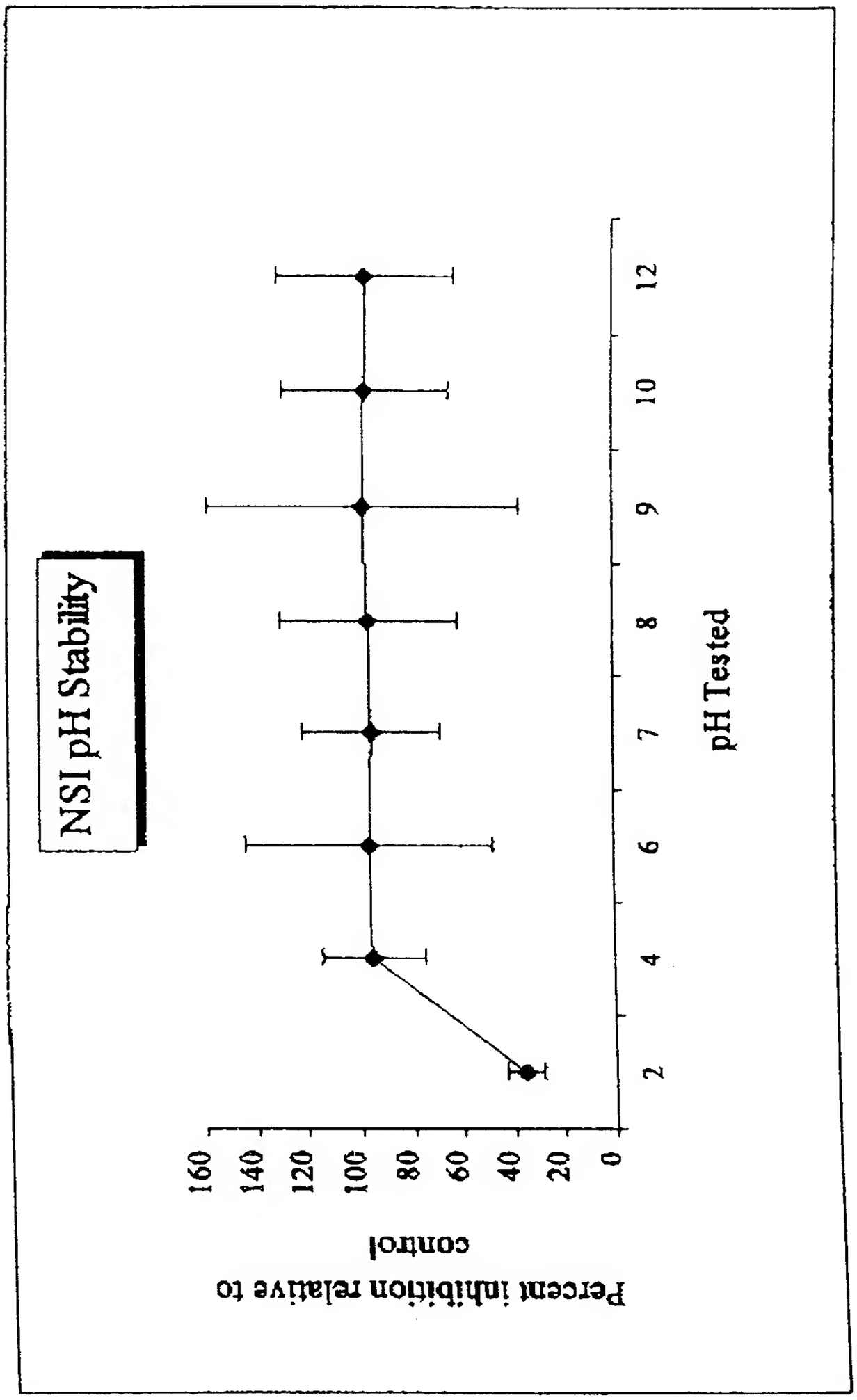
NSI pH Stability
Percent inhibition relative to control
160
140
120
100
80
60
40
20
0
2
4
6
7
8
9
10
12
pH Tested

FIGURE 5A

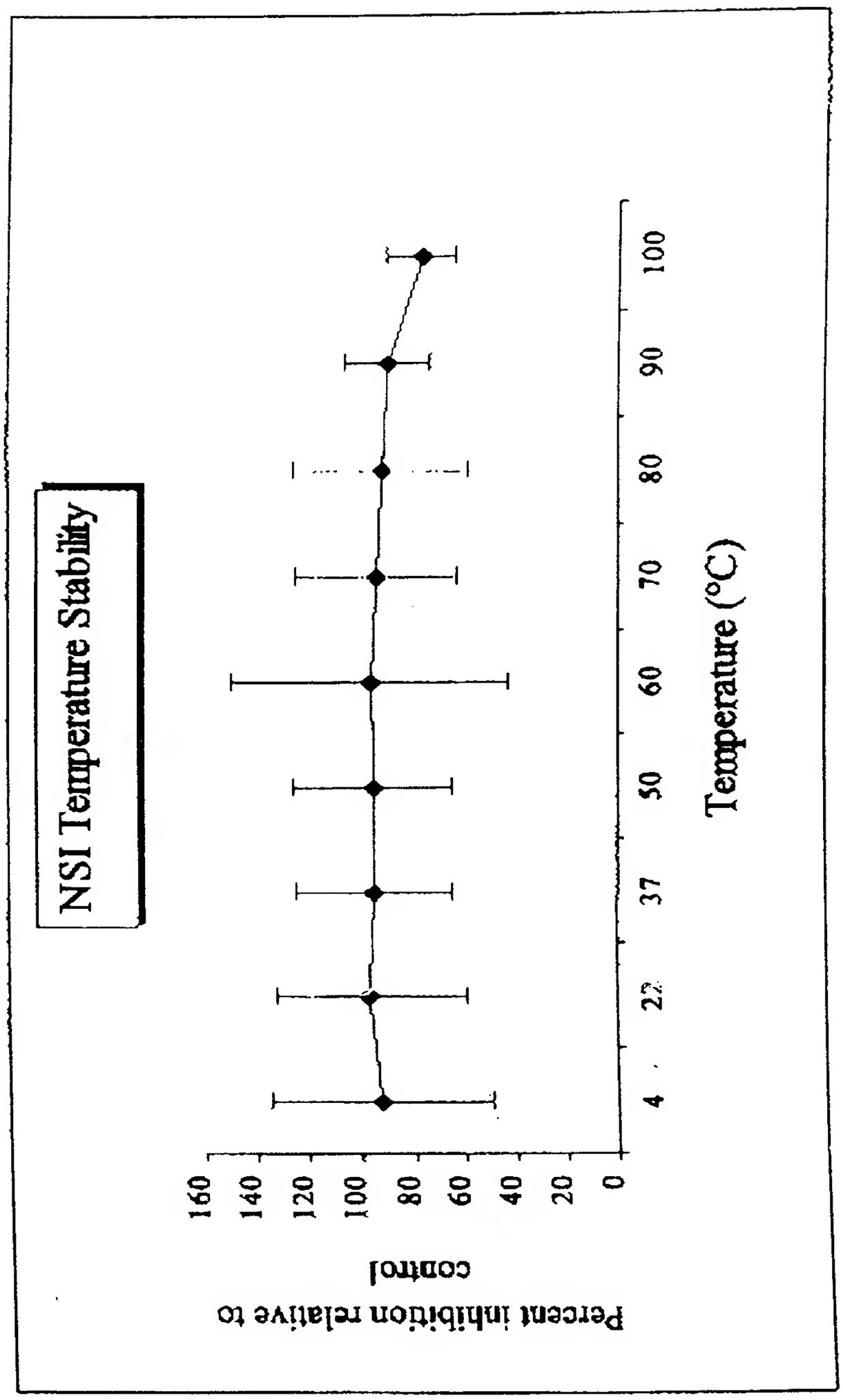
NSI Temperature Stability
Temperature (°C)
4
22
37
50
60
70
80
90
100
Percent inhibition relative to control
0
20
40
60
80
100
120
140
160

FIGURE 5B

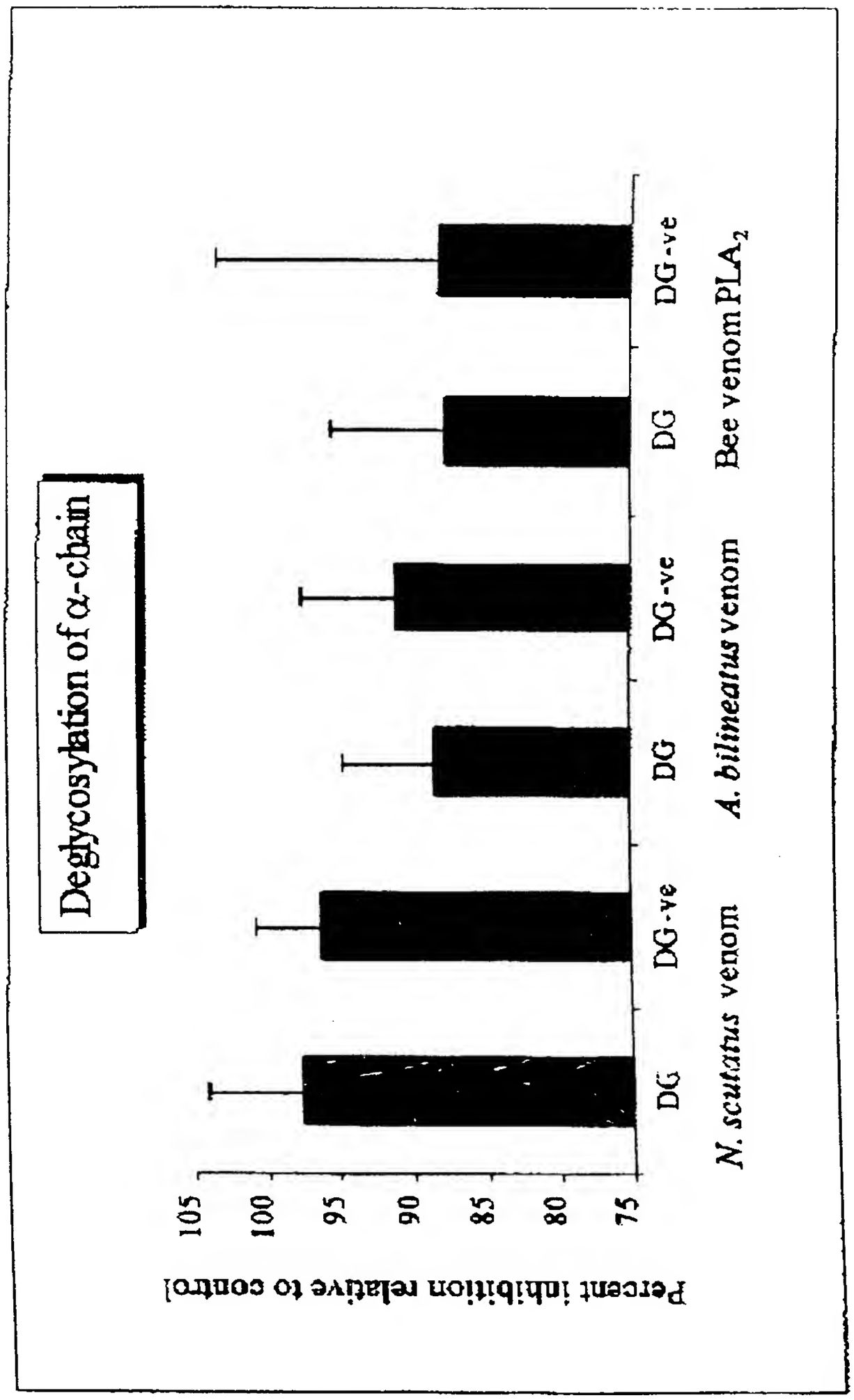
Deglycosylation of α-chain
Percent inhibition relative to control
105
100
95
90
85
80
75
DG
DG-ve
DG
DG-ve
DG
DG-ve
N. scutatus venom
A. bilineatus venom
Bee venom PLA2

FIGURE 6A

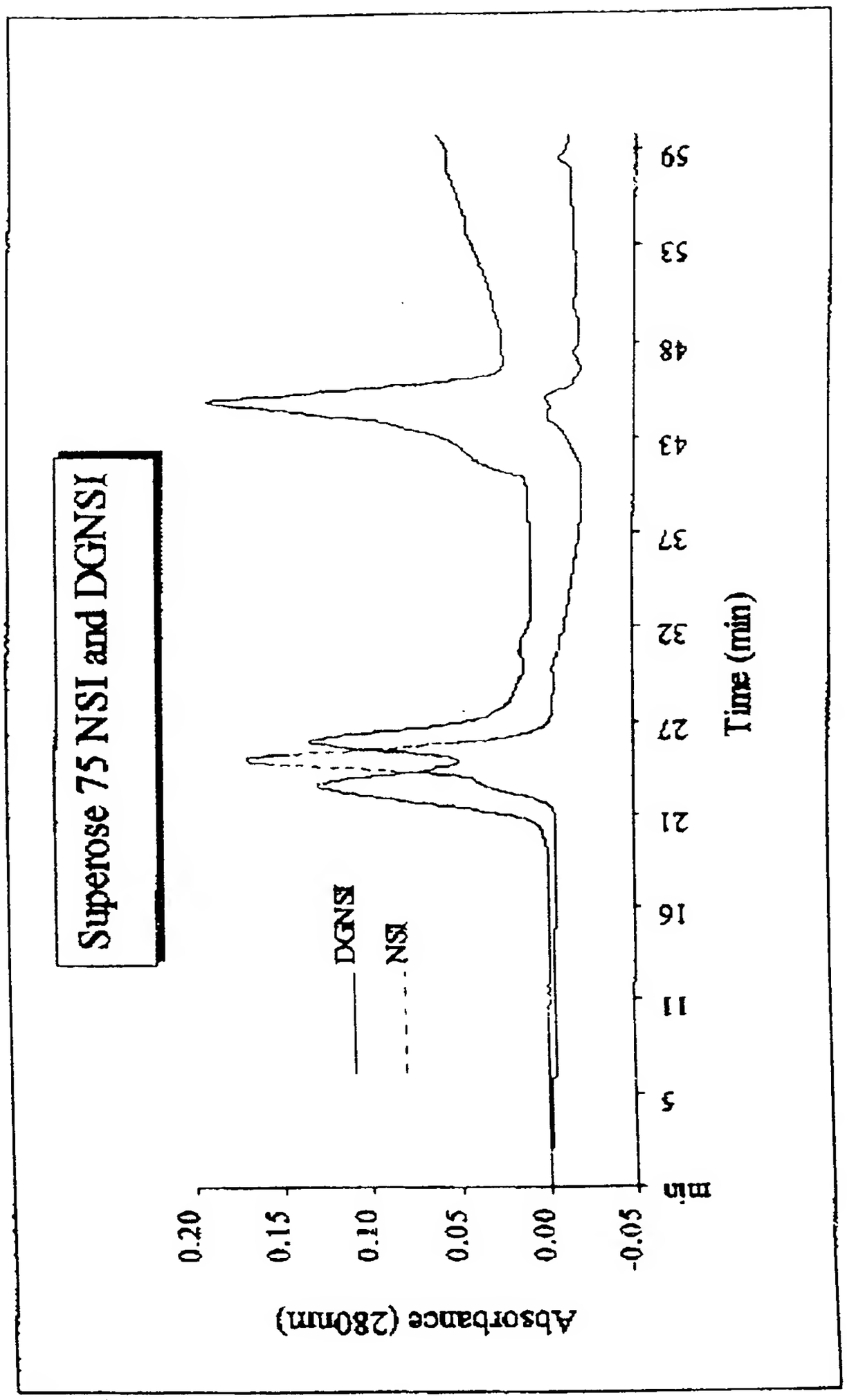
Superose 75 NSI and DGNSI
DGNSI
NSI
Absorbance (280nm)
0.20
0.15
0.10
0.05
0.00
-0.05
mls
5
11
16
21
27
32
37
43
48
59
53
Time (min)

FIGURE 6B

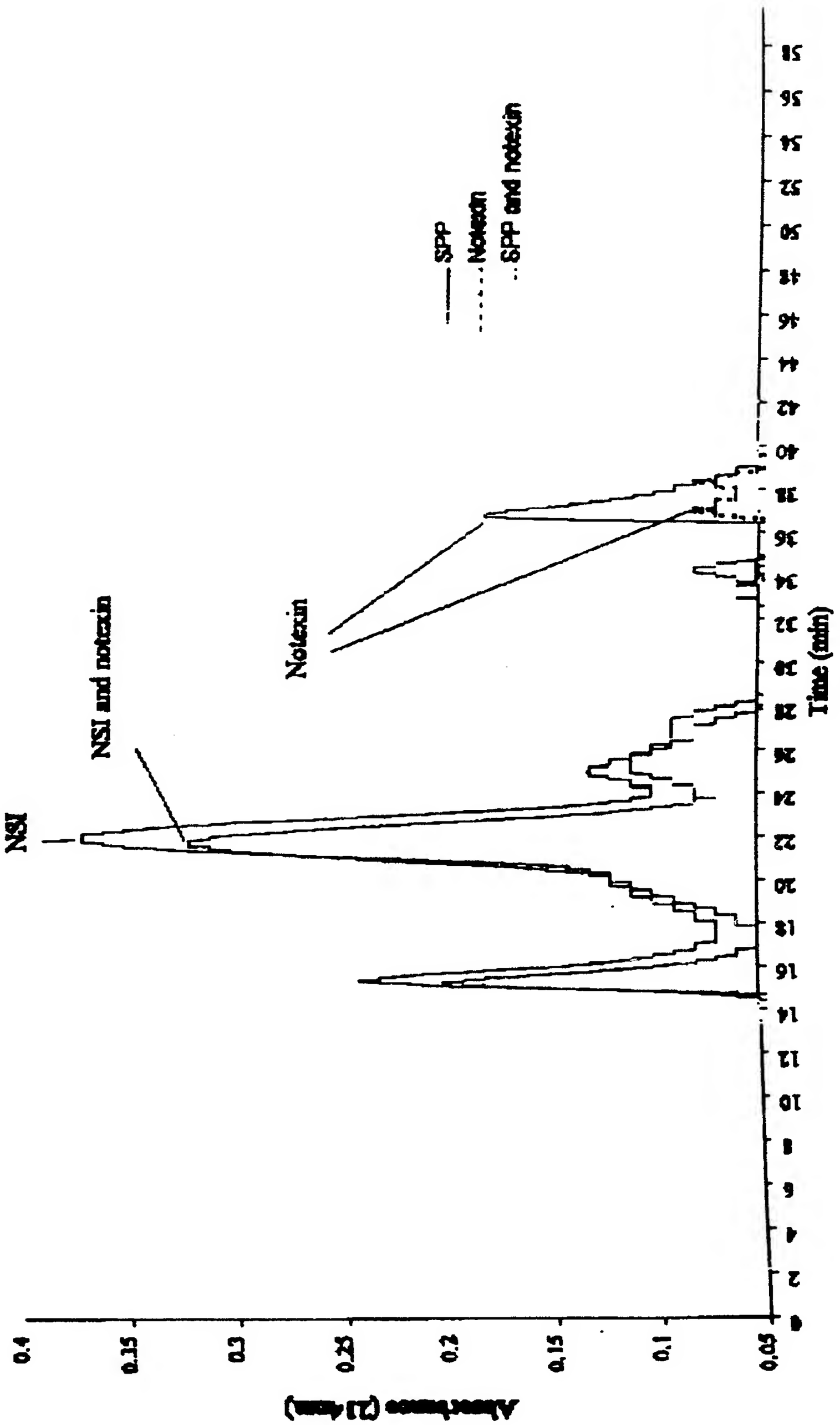
SPP
Notexin
SPP and notexin
NSI
NSI and notexin
Notexin
Time (min)
Absorbance (214 nm)

FIGURE 7

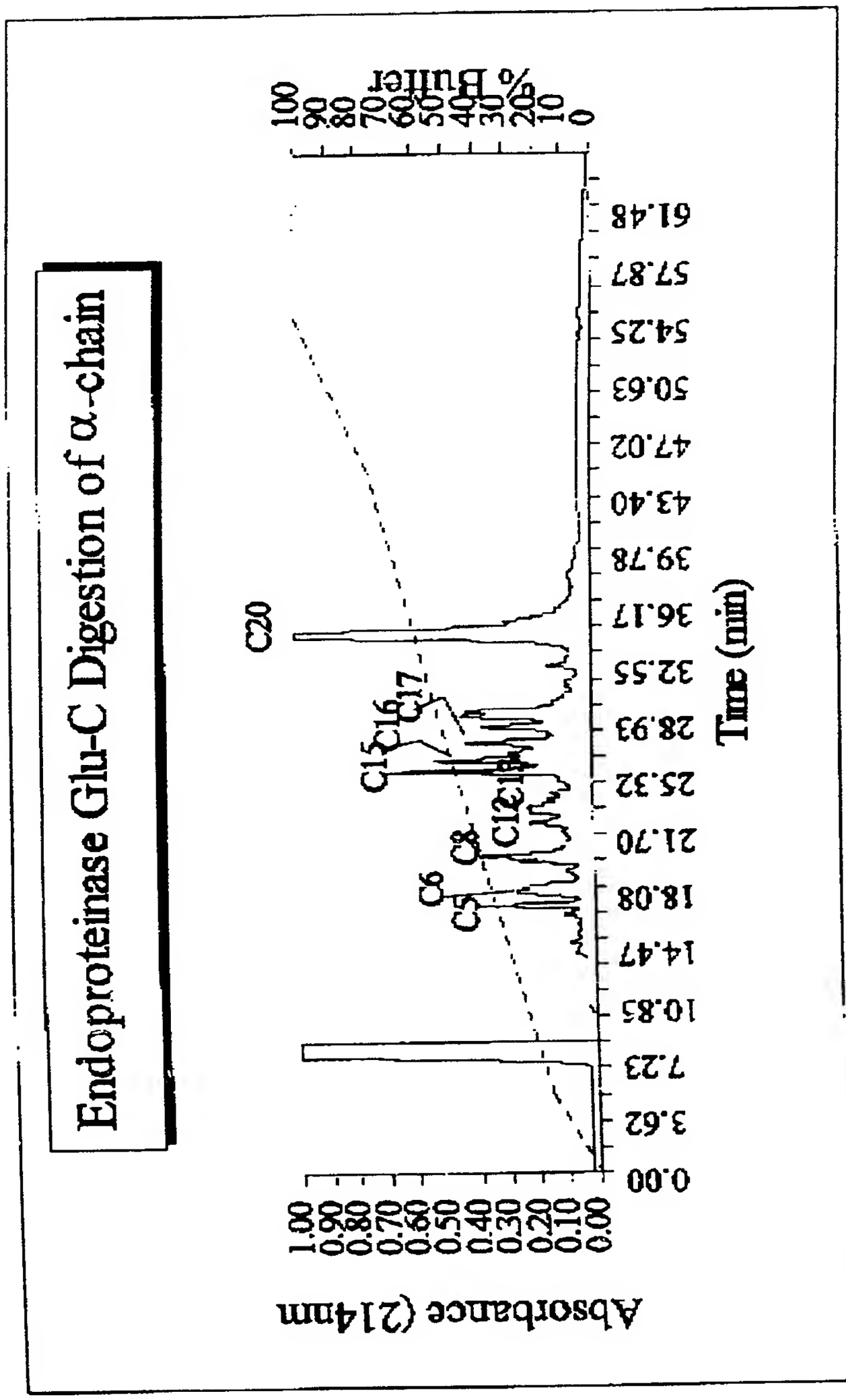
Endoproteinase Glu-C Digestion of α-chain
Absorbance (214nm
Time (min)
% Buffer
C20
C15
C16
C17
C6
C5
C8
C12

FIGURE 8

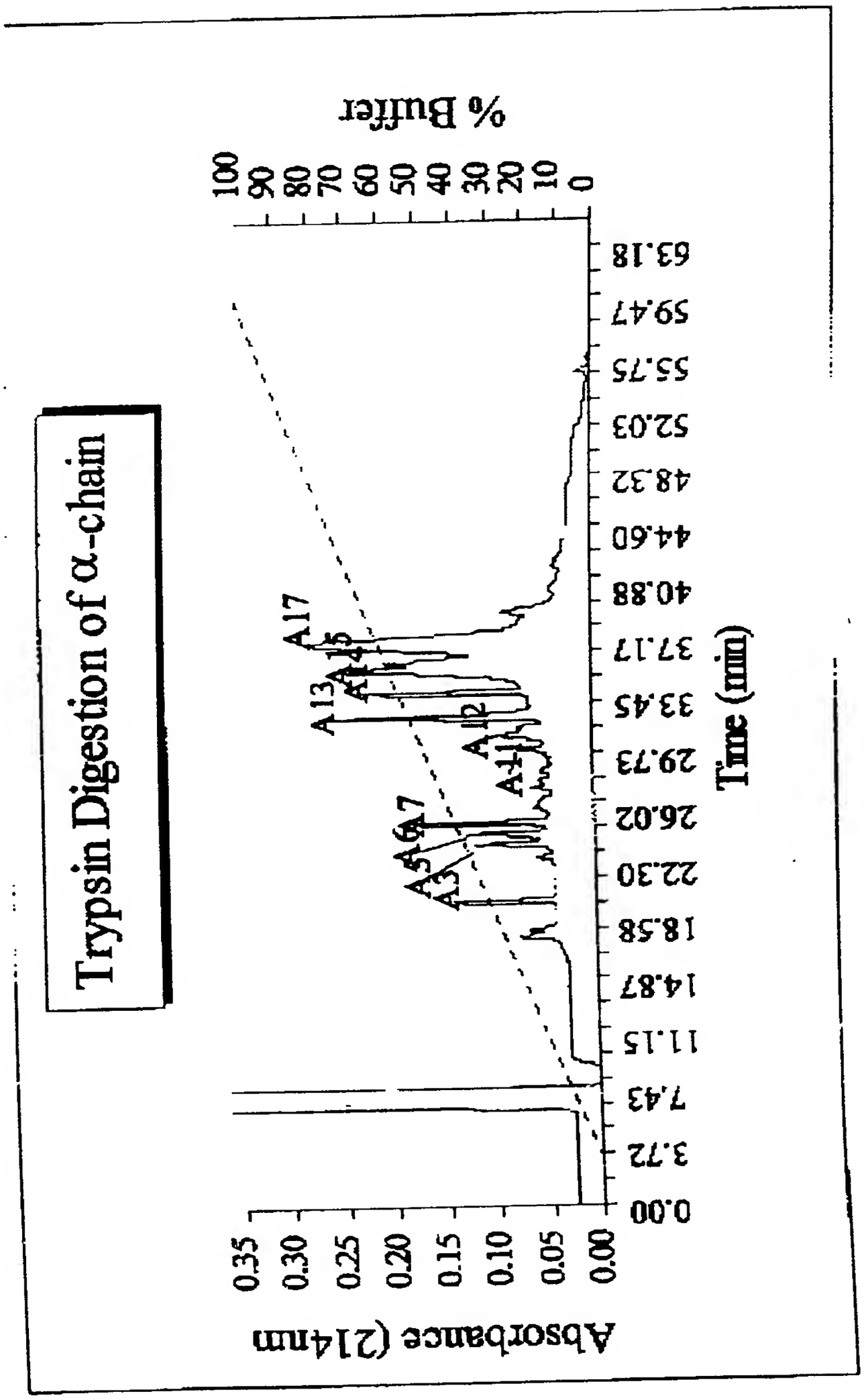
Trypsin Digestion of α-chain
% Buffer
100 90 80 70 60 50 40 30 20 10 0
63.18
59.47
55.75
52.03
48.32
44.60
40.88
37.17
33.45
29.73
26.02
22.30
18.58
14.87
11.15
7.43
3.72
0.00
Time (mins)
A17
A13
A15
A14
A12
A11
A7
A6
A5
A3
0.35 0.30 0.25 0.20 0.15 0.10 0.05 0.00
Absorbance (214nm

FIGURE 9

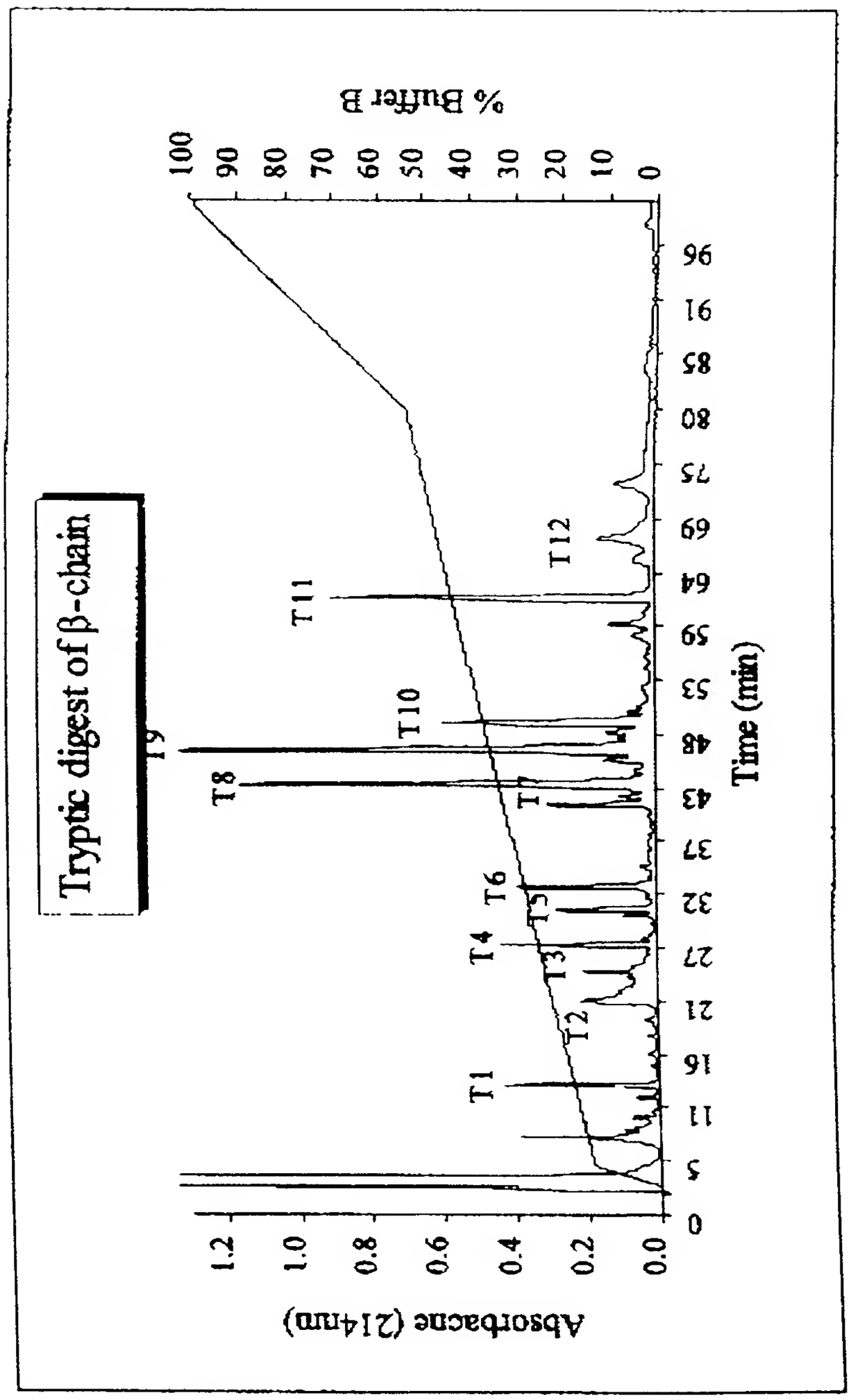
% Buffer B
100
90
80
70
60
50
40
30
20
10
0
96
91
85
80
75
69
64
59
53
48
43
37
32
27
21
16
11
5
0
Time (min)
Tryptic digest of β-chain
T9
T8
T11
T10
T12
T7
T6
T5
T4
T3
T2
T1
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Absorbacne (214nm)

FIGURE 10

```
proseqnsi1      MKSLQIICLLFVLVARGSCHSCEICHNLGRDCETEEAEECASPEDQCGTVLMEVSSAPIS 60
pseqct1         MISLQIICFLFVLVARGSCHSCEICRNFGKDCESEEAEECASPEDQCGTVLLEISSAPIS 60
pseqit1         MKSLQIICPLFVLVARGSCRSCEICHNFGKDCESEEAEECASPEDQCGTVLLEISSAPIS 60

proseqnsi1      FRSIHRNCFSSSLCKLERFDINIGHDSYLRGRIHCCDEARCEAQQFPGLPLSFPNGYHCP 120
pseqct1         FRSIHRNCFSSSLCKLEHFDINIGHDSYVRGRIHCCDEERCEAQQFPGLPPSLPNGYHCP 120
pseqit1         FRSIHRNCFSSSLCKLEHFDINIGHDSYVRGRIHCCDEERCEAQQFPGLPLSFPNGYHCP 120
```

FIGURE 11(CONT.I)

```
proseqnsi1      GILGVFSVDSSEHEAICRGTETKCINLAGFRKERFPGDIAYNIKGCTSSCPELRLSNRAH 180
pseqct1         GILGAFSVDSSEHEAICRGTETKCINLAGFRKERYPVDIAYNITGCTSSCPELKLSNRTH 180
pseqit1         GILGAFSVDSSEHEAICRGTETKCINLAGFRKERYPVDIAYNIKGCTSSCPELKLSNRTH 180

proseqnsi1      EEDRNDLIKVECTDASKITPSEI 203
pseqct1         AERRNALITLDCTDASKIAPSE- 202
pseqit1         EERRNDLITLECTDASKITPSE- 202
```

FIGURE 11(CONT. II)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 98/00992

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C07K 14/46, 19/00; C07H 21/04; A61K 38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN, FILE REG, SUBSEQUENCE SEARCH; ANGIS, BLAST P, FAST A, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98/13376 A, (GARVAN INSTITUTE OF MEDICAL RESEARCH), 2 April 1998. See whole document	1-3, 10, 15 16, 35
X	Biochem.Biophys. Res. Commun., 204, 1212-1218, (1994), N. Ohkura *et al.*, "The two subunits of a Phospholipas A_2 Inhibitor from the Plasma of Thailand Cobra..."	1-5, 7-13 15, 16, 35
X	Eur. J. Biochem., 249, 838-845 (1997), I. Nobuhisa *et al.*, "Characterisation and evolution of a gene encoding a *Trimeresurus flavoviridis* serum protein..."	1-5, 7-13 15-32, 35, 36

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 December 1998	21 JAN 1999
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929	Authorized officer **L.F. McCAFFERY** Telephone No.: (02) 6283 2573

INTERNATIONAL SEARCH REPORT

	International application No. PCT/AU 98/00992

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Hoppe-Seyler's Z. Physiol. Chem., 360, 1075-1090, (1979), F. J. Joubert *et al.*, "The Amino Acid Sequence of the Subunits of two reduced and S-carboxymethylated proteins..."	1-3, 7-13 15, 16, 35
X	J. Biol. Chem., 269(22), 15646-15651, (1994), C. L. Fortes-Dias *et al.*, "A Phospholipase A_2 Inhibitor from the Plasma of the South American Rattlesnake (*Crotalus durissus terrificus*)."	1-5, 7-13 15-32, 35, 36
X	Medline Abstract PMID 7851385, Eur. J. Biochem., 227, 19-26, (1995), J. Perales *et al.*, "Molecular Structure and Mechanism of action of the crotoxin inhibitor from *Crotalus durissus terrificus* serum."	1-5, 7, 8, 10 15, 16, 35, 36

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 98/00992

Box 1 Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: **1-8, 17-22, 36**

because they relate to subject matter not required to be searched by this Authority, namely:

These claims are drafted in such a way as to make a complete search impossible on economic grounds. Accordingly the search has been substantially limited to sequences disclosed in the specification.

2. [ ] Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU 98/00992

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
WO	98/13376	AU	43712/97

END OF ANNEX